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ON DETERMINATIVE CLEAVAGE AND YOLK FORMATION IN THE HARPACTID COPEPOD TISBE FURCATA (BAIRD)

EMIL WITSCHI

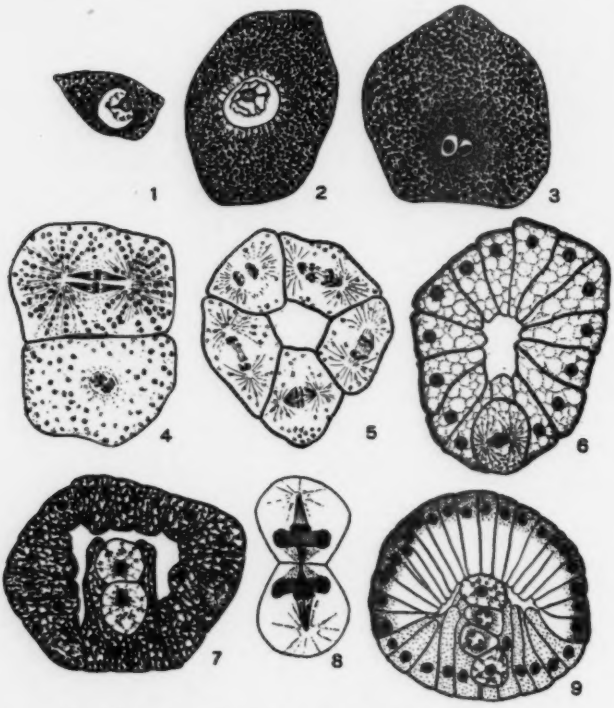
(From the Zoölogical Laboratory, State University of Iowa, and the Marine Biological Laboratory, Woods Hole, Mass.)

The paper by Grobben, in 1879, on the development of the crustacean *Moina rectirostris* initiated that long series of investigations on cell lineage and on formative substances which culminated in the experimental work of Boveri, F. R. Lillie, E. B. Wilson, Conklin, and many other distinguished embryologists. The crustacean egg itself, while too delicate for most experimental purposes, has been found interesting and favorable for cytological observation. Thus Kühn (1913) was able to trace the origin of the germ cells in the cladoceran *Polyphemus* from the very first cleavage division, the "Keimbahn" being marked by a cell inclusion that forms from nurse-cells during the later stages of ovogenesis. Häcker (1897) and his student Amma (1911) observed in the normal development of several species of copepods the presence of a special type of granules which collect at one end of the mitotic spindle during the early cleavage divisions. Consequently, after the fifth division the granules are transmitted to only one of the 32 blastomeres. This stem cell next divides into a primordial endoderm and a primordial germ cell, of which the latter receives most or all of the characteristic granules. These "ectosomes" of Häcker, in the present writer's opinion, may well be classed as mitochondria if the latter term is not given a too narrow and specialized definition.

Some years ago I found in one of my aquaria, among algæ collected along the shore of the harbor of Woods Hole, numbers of a large harpactid which were mostly females with egg sacs. I am indebted to Charles B. Wilson (1932) for his assistance in the identification of the species as *Tisbe furcata* (Baird). The embryological study revealed some peculiarities which seem to justify the publication of this short account.

The adult female of *Tisbe furcata* has paired ovaries which fuse

with their anterior ends, dorsally above the intestine of the thoracic region (similar condition reported by Heberer (1930) for *Eucalanus*). Yolk formation in the ovocytes starts quite early (Fig. 1), and during



Camera lucida drawings. Fig. 8, × 1,000; all others × 500.

FIGS. 1 and 2. Ovarial ovocytes.

FIG. 3. Newly deposited and fertilized egg from the brood sac. Note the yolk granules (black) which have attained the maximal size for the egg yolk.

FIG. 4. Second cleavage division. Yolk not represented. In this and the two following figures note the flakes of mitochondria in the dividing cells.

FIG. 5. Fifth cleavage division. Yolk not represented.

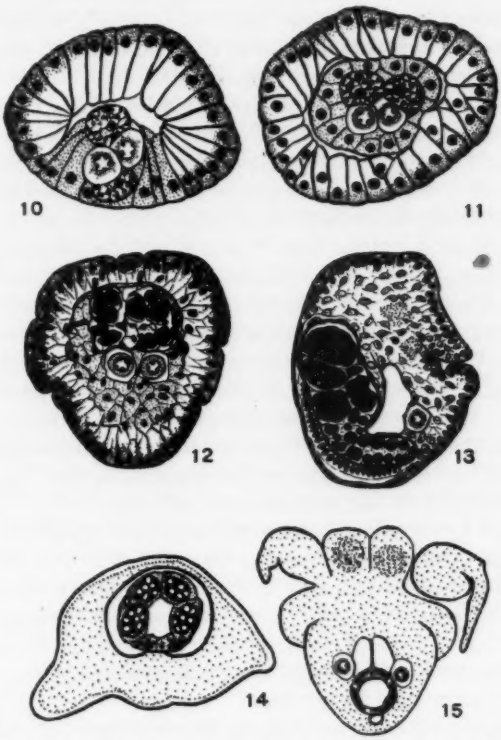
FIG. 6. Blastula of 63 cells. The stem cell near the vegetative pole just completing the sixth cleavage division. Yolk not represented.

FIG. 7. Early gastrula. Polar ingrowth of mesoderm cells carrying along the two stem cells. Yolk granules small; few in the stem cells, numerous in all other cells.

FIG. 8. Simultaneous differential division of the two stem cells. Spindles attached to the inner cell walls. Mitochondrial rings around the inner spindle cones.

FIG. 9. Gastrula. Ectodermal and mesodermal regions indicated by different stippling. Each stem cell has divided into an endodermal cell (with small yolk granules) and a primordial germ cell (with mitochondria, and vesicular nuclei). The four cells form an axial row.

the later growth of the eggs the platelets increase only in number but not in size (Fig. 2). After fertilization the characteristic double nuclei form (Fig. 3) and the first cleavage divisions are distinguished by the formation of double spindles and the maintenance of separate paternal and maternal groups of chromosomes (Fig. 4). During the mitotic



FIGS. 10, 11. Completion of gastrulation and rearrangement of the endoderm and germ cells. Yolk begins to accumulate in the endoderm cells. Ectoderm and mesoderm indicated as in Fig. 9.

FIG. 12. Frontal section through embryonic nauplius. Large yolk granules (black) in the endoderm. Two germ cells with vesicular nuclei and mitochondrial shell.

FIG. 13. Sagittal section through slightly older embryo. Extremely large yolk bodies in anterior part of the endoderm, smaller ones in the posterior section. Body cavity and ganglia are formed. Only one germ cell visible.

FIG. 14. Cross-section through nauplius near hatching stage. Yolk bodies in the walls of the gut become smaller.

FIG. 15. Frontal section through nauplius at time of hatching. Endodermal yolk nearly resorbed. The two gonocytes left and right of the body cavities.

process flakes of mitochondria appear (Figs. 4 and 5) which, however, are evenly distributed within the blastomeres. Toward the end of the sixth cleavage division one cell becomes conspicuous by its round shape and retarded division. Figure 6 shows this 63-cell stage with the round "stem cell" still in the metaphase of the sixth division. The mitochondria are evenly distributed and two equipotent daughter cells result, which have the value of left and right "stem cells." While the embryo passes through the seventh and eighth cleavage divisions gastrulation by polar immigration takes place (Figs. 7, 9, 10, 11). The "stem cells" are carried to the center of the embryo by the ingrowth of the mesoderm. Temporarily, they change from the bilateral to an axial orientation (Fig. 7). At that time the resorption of the yolk has made considerable progress. Only small granules remain in the protoplasmic meshwork. They are especially scarce in the two "stem cells" (Fig. 7). The next division is of great interest since it is qualitatively unequal, giving rise to a pair of primordial endoderm and a pair of primordial germ cells (Fig. 9). Figure 8 shows how the mitochondria, which had remained visible during the previous interkinetic phase (Fig. 7), become condensed and form rings around the inner halves of the spindles of the "stem cells." These rings are attached to the inner spindle poles by fine fibers. The subsequent unequal cell division results in the formation of two small inner cells which contain the mitochondria, and two large outer cells which contain most of the yolk granules (Fig. 9). The former are the primordial germ cells, the latter the first endoderm cells.

During the later phase of gastrulation the four cells resume a bilateral arrangement (Figs. 10, 11), the endodermal cells occupying a position antero-dorsal to the germ cells. The germ cells remain without further divisions up to the time when the nauplius larva hatches from the egg sac (Fig. 15). They are easily identified by their large size, large nuclear vesicle with compact chromosomes, and by the dense shell of mitochondria around the nucleus.

Of great interest is the behavior of the endoderm cells, which multiply by mitotic division and give rise to the midgut, as indicated in Figs. 12 to 15. During the early phase of nauplius differentiation they exhibit a peculiar process of yolk formation (Figs. 11–13). The rapidly growing yolk platelets soon begin to coalesce, resulting in the formation of about eight large and many small grains. Some of the endodermal cells become greatly inflated, assuming the function of a dorsal yolk sac in the embryonic nauplius (Fig. 13). Recent students of yolk differentiation have concentrated their attention on the synthesis which takes place during ovogenesis. Although given little attention, endodermal yolk formation as observed in *Tisbe* is widespread among crustaceans.

It is closely related to the " vitellophage " process by which the endoderm of the crayfish *Potamobius* absorbs the uncleaved yolk mass of the egg (Reichenbach, 1886). The only difference is that in the former species the *egg yolk* during cleavage is evenly distributed in all blastomeres. As in the crayfish this primary yolk becomes liquified and when redeposited as *endodermal yolk* it forms grains of strikingly different size. It may be added that the endodermal yolk gives the same reactions with eosin, acid fuchsin, iron hematoxylin, and other dyes as the yolk of the ovarial egg and the early blastomeres.

If compared with cladocerans the development of the copepods shows many striking similarities. However, the " stem cell " appears at the close of the third cleavage division (Grobben, 1879; Kühn, 1913) and divides during the fourth into one endoderm and one germ cell. According to McClendon's (1907) description of the development of parasitic copepods, it seems that the " stem cell " is formed at the close of the fourth cleavage. In non-parasitic copepods, however, the " stem cell " is formed only by the fifth cleavage. In *Cyclops fuscus, Diaptomus cæruleus,* and other species investigated by Amma (1911) the sixth division has been found to be segregational, while in *Tisbe furcata* it produces a pair of equal left and right " stem cells." The seventh division consequently is equal in *Cyclops* and *Diaptomus,* but segregational in *Tisbe,* thus producing in all genera one pair each of endoderm and germ cells. The postponement of the segregational division in *Tisbe* coincides with the practically equal distribution of mitochondria in the early cleavage stages of this species. We do not intend to reopen the discussion about the significance of the mitochondria or " ectosomes." However, it is evident that mitochondria by their peculiar distribution, if not responsible directly for the segregation of endoderm and germ plasm, indicate at least a polar differentiation within the cytoplasm of the stem cells, even before the beginning of the differentiating division.

Summary

In the development of *Tisbe furcata,* a harpactid copepod of the Woods Hole region, there occur two periods of yolk formation. Egg yolk is accumulated in the usual way during ovogenesis. After gastrulation and during the differentiation of the nauplius, yolk is deposited again in the endoderm, which partly assumes the character of a yolk sac.

As in other non-parasitic copepods, endoderm and germ cells originate from a common " stem cell " of the 32-blastomere stage. *Tisbe furcata* is peculiar in that an equational division precedes the one that separates the endodermal from the germinal line. In the stem cells the

segregational division is prepared by a polar differentiation of the cytoplasm which is most clearly indicated by the asymmetrical distribution of the mitochondria.

BIBLIOGRAPHY

AMMA, K., 1911. Über die Differenzierung der Keimbahnzellen bei Copepoden. *Arch. f. Zellf.*, **6**: 497.

GROBBEN, C., 1879. Die Entwicklungsgeschichte der Moina rectirostris. *Arb. Zool. Inst. Wien*, **2**: 203.

HÄCKER, V., 1897. Die Keimbahn von Cyclops. *Arch. mikr. Anat.*, **49**: 35.

HEBERER, G., 1930. Die Struktur der Oocyten von Eucalanus elongatus Dana mit Bemerkungen über den Bau des weiblichen Genitalapparates. *Zeitschr. wiss. Zool.*, **136**: 155.

KÜHN, A., 1913. Die Sonderung der Keimesbezirke in der Entwickelung der Sommereier von Polyphemus pediculus de Geer. *Zool. Jahrb. (Anat. u. Ont.)*, **35**: 243.

MCCLENDON, J. F., 1907. On the Development of Parasitic Copepods. *Biol. Bull.*, **12**: 53.

REICHENBACH, H., 1886. Studien zur Entwicklungsgeschichte der Flusskrebses. *Abh. Senckenberg. Nat. Ges. Frankfurt*, **14**: 1.

WILSON, CH. B., 1932. The Copepods of the Woods Hole Region, Massachusetts. *Bull. U. S. Nat. Mus.*, **158**: 1.

FURTHER OBSERVATIONS ON THE POLARITY OF THE EGGS OF URECHIS CAUPO

VESTA HOLT

(From the Hopkins Marine Station, Stanford University)

The eggs of *Urechis caupo,* an echiuroid worm recently described by Fisher and MacGinitie (1928), are found in abundance at several readily accessible points along the coast of California. Owing to their abundance, size, and transparency, and to their unusual viability under ordinary laboratory conditions, they are proving to be valuable material, especially for the experimental embryologist. It is therefore essential that their normal embryology be known, and of first importance that a definite mark of polarity in the immature eggs, if present, be recognized.

The results of a series of checks upon the polarity of normal and centrifuged eggs of *Urechis,* with the conclusion that the immature egg bears no visible mark of polarity, were published by Taylor in 1931. Earlier in the same year, Tyler (1931) presented evidence that the indentation, which is characteristic of these eggs, marks the animal pole. Later, this author (Tyler, 1932) published further data and concluded that " The main question involved in this work is whether or not the polarity of the *Urechis* egg is determined before fertilization, as appears to be the case for most animal eggs. For the type first discussed the results clearly show that polarity is already established in the unfertilized egg, and the pole is marked by the position of the indentation. For the type in which a second indentation appears after fertilization there is no reason to doubt that polarity is established before fertilization although the position of the pole is not so clearly marked by the first indentation as by the one appearing after fertilization."

According to this author, then, there are two types of eggs in *Urechis* as regards the point of extrusion of polar bodies: (1) those wherein this extrusion coincides with an indentation *before* fertilization, and (2) those in which, *after* fertilization, a second indentation forms and corresponds with the point of extrusion of polar bodies. Just what relation this second indentation has to the one in immature eggs is not made clear so that immature eggs of this second type apparently have no mark of polarity.

Because of the discrepancies between our findings of 1931 and those

of Tyler (1931, 1932), we undertook to recheck our former results and to make further studies on the polarity of this egg for comparison with Tyler's data.

For this purpose, eggs were used from fourteen females. Three of these, viz., 1, 2, and 3 (see Table I), which were used during July and August, 1931, were dug at Morro Bay on July 18, then taken directly to Pacific Grove in a glass jar of sea water which was kept cool in a bath of ice water, and placed in aquaria of running sea water. The studies on the eggs of these females were made from July 20 to August 10.

The eleven females, *A* to *K* inclusive, and the male *A* were dug within an area of one square yard at Elkhorn Slough on December 18, 1931. These were taken to the laboratory in jars containing water from the slough and transferred within two hours to aquaria with running sea water. The *ME* female was one of the three from Morro Bay which had been used the previous summer and left in the aquarium of running sea water until December, when further studies were made on the eggs, as indicated in Table I.

The vaseline slide method recommended by Tyler (1931) was used. For recording the location of polar bodies, the surface of the egg was roughly divided into three regions. Region I is the 90° containing the indentation and corresponding approximately to its size; Region II is the equatorial band; and Region III comprises the 90° of area opposite the indentation. Also a check was made of the eggs which developed polar bodies within 10° of the center of the indentation.

Many eggs show two or more indentations. It may be that many of those considered above have another indentation up or down, but none was recorded which showed more than one. In the following tabulation, polar body formation in eggs showing two indentations is recorded. These eggs were taken from various females and the check made along with the previous checks.

In the smaller indentation	25	eggs	38%
In the larger indentation	10	"	15%
In neither indentation	31	"	47%
Total	66	eggs	100%

Microphotographs which were made of many of these eggs definitely show the same points listed in these tables.

In June, 1934, a more carefully controlled study was made on a small number of eggs. Each egg was rolled over by means of a fine jet of water from a micropipette and the entire surface examined. Only those eggs were chosen which showed one indentation. Each egg was kept under *constant* observation for the first 15 minutes after insemination

and in several cases until polar body formation. The slides were never removed from the microscope until after maturation.

Two groups of females were used. The first group had been dug at Elkhorn Slough May 10 and had been used during the interim to supply eggs for other experiments. The females of the second group were also from the slough but were freshly dug at the time of the study, June 17. The results are given in Table II.

TABLE I

Females	Dates	Temp. range °C.	No. eggs with polar bodies in Region I	No. eggs with polar bodies in Region II	No. eggs with polar bodies in Region III	No. eggs 0°–10° divergence	No. eggs 10°–180° divergence
1	7/20–8/6	18½–20½	58	19	9	44	40
2	8/5–8/10	19 –20½	25	46	11	17	65
3	8/6	19½–20½	2	5	3	1	9
A	12/21, 29	15 –18	20	37	10	11	52
B	12/22–1/1	14½–18½	17	27	14	9	49
C	12/23	14 –16	6	6	5	2	17
D	12/24	15½–17½	6	6	3	3	13
E	12/24	17 –17½	4	11	3	4	14
F	12/25	16 – 16	3	13	2	1	17
ME	12/26, 27	16 –18½	12	11	7	11	20
G	12/26, 31	13 –17½	8	7	2	9	15
H	12/28	18 –19½	3	15	3	2	20
I	12/29	15½–16½	13	13	3	10	19
J	12/30	18 –18½	4	12	1	5	13
K	12/31	17 –18	7	4	1	6	6
Totals			188	232	77	135	369
Percentage of total number of eggs			38	47	15	27	73

Careful observation of the egg following insemination reveals a fairly regular cycle of events. Original indentations disappear between

2 and 4 minutes, the membrane appears in approximately 5 minutes, and secondary indentations appear from 6 to 9 minutes. There may be *one or many* of these secondary indentations. They may appear within the

TABLE II

	Old Worms	Fresh Worms
Polar body forms in original indentation	10	2
Polar body forms in a secondary indentation within area of original	2	3
Polar body not in area of original indentation but		
1. Where there has been no indentation at any time	1	1
2. Where a secondary indentation was observed to form after insemination	5	8
3. On the upper or lower surface of the egg where there was no original indentation but where a secondary may or may not have formed	3	7

area of the original or at any point on the surface of the egg. They usually remain from 4 to 9 minutes. Fifteen out of forty eggs formed polar bodies at the location of a secondary indentation, but thirty-eight such indentations (in twenty eggs) were observed which bore no relation to the point of extrusion of polar bodies.

From these observations it appears that the behavior of the indentations in *Urechis* eggs may be a purely physical phenomenon, related to permeability and viscosity changes following fertilization. Centrifuging results show a marked fall in viscosity at fertilization. This is no doubt due to the absorption of water which causes the indentations to fill out. Then there is a reversed process. Measurements of eggs show that at fertilization there is only a slight membrane elevation but that the perivitelline space results primarily from a shrinkage of the egg away from the membrane. Accompanying this shrinkage are numerous bucklings and the formation of temporary, secondary indentations over the surface of the egg.

Whatever the explanation for these various changes on the egg surface may be, two points are evident from the results published by Taylor in 1931, those of Tyler in 1932, and those given above:

1. That polar bodies do not *always* appear at an indentation found in the immature egg. Hence this cannot be used as a mark of polarity.
2. That polar bodies do not appear at all secondary indentations of the fertilized egg. Hence a specific, secondary indentation cannot be used as a reliable mark of polarity, even *after* fertilization.

Therefore we maintain our original thesis, viz., that there is no reliable mark of polarity on the immature egg of *Urechis caupo* and until such

a mark can be located with certainty, it cannot be said that the polarity of this egg is, or is not, established prior to fertilization.

LITERATURE CITED

FISHER, W. K., AND G. E. MACGINITIE, 1928. A New Echiuroid Worm from California. *Ann. and Mag. Nat. Hist.*, Ser. 10, **1**: 199.

TAYLOR, C. V., 1931. Polarity in Normal and Centrifuged Eggs of Urechis caupo Fisher and MacGinitie. *Physiol. Zool.*, **4**: 423.

TYLER, ALBERT, 1931. The Production of Normal Embryos by Artificial Parthenogenesis in the Echiuroid, Urechis. *Biol. Bull.*, **60**: 187.

TYLER, ALBERT, 1932. The Polarity of the Egg of Urechis caupo. *Biol. Bull.*, **63**: 145.

ACCESSORY HEARTS IN THE OYSTER, OSTREA GIGAS[1]

A. E. HOPKINS

U. S. BUREAU OF FISHERIES

It is well known that in some invertebrates certain peripheral blood vessels are specialized to pulsate and pump blood to organs which otherwise would receive very little circulation. The pulsating ampullae at the bases of the antennae of some insects are frequently referred to as examples of peripheral accessory hearts. (See von Buddenbrock, 1928.) More widely recognized are the "gill hearts" of cephalopods, though it appears that similar structures have not been identified in other mollusks.

During a study of the mechanism by which the Japanese oyster (*Ostrea gigas*) pumps water, a number of specimens were prepared for observation by sawing away portions of the right, or upper, valve. Generally the free edge of the mantle folded back over the cut surfaces, where it started to secrete new shell. In such cases the mantle chamber, in which the gills hang, and the cloacal chamber, from which exhalant water is discharged, were exposed. In these oysters a pair of well-defined, actively pulsating vessels was observed. It appears that, due to failure to observe these organs, some authors have not understood the circulatory system of the oyster entirely correctly.

In the expanded condition the accessory hearts appear as prominent vessels along the inner surfaces of the mantle folds which form the lateral walls of the cloacal chamber (Fig. 1). Distally they end near the margin of the mantle, slightly forward of the septum which connects the two mantle lobes and to which the posterior ends of the gills are attached. Centrally they disappear into the excretory organs (organ of Bojanus) adjacent to the ventro-lateral borders of the adductor muscle. In this manner they cross the cloacal chamber in a diagonal direction.

Dissections after injection showed that apparently a thin septum separates the blood spaces of the excretory organ from veins entering the auricles. If, as appears from these observations, the spaces of the kidney do not open directly into the auricles but are forced to dispose of the blood collecting in them from the viscera in some other manner, it is necessary to modify our concept of the mode of circulation. The gills are apparently supplied only with venous blood from the organ of Bojanus while such blood enters the mantle along with arterial blood.

[1] Published by permission of the Commissioner of Fisheries.

It was stated by Leenhardt concerning the Portuguese oyster, *Gryphea* (*Ostrea*) *angulata* (1926, p. 82) that: "Les branchies ne reçoivent que du sang provenant de l'organe bojanien, mais les lacunes de cet organe communiquent avec une grosse veine afférente du coeur qui peut ramener directement le sang à l'oreillette sans passer par la branchie." He considered that the circulatory system of the gills is "adrift" on the lacunae of the excretory organ which has a much larger vessel leading into the heart, so that there would be little pressure to force blood through the gills. However, he did not observe the accessory hearts.

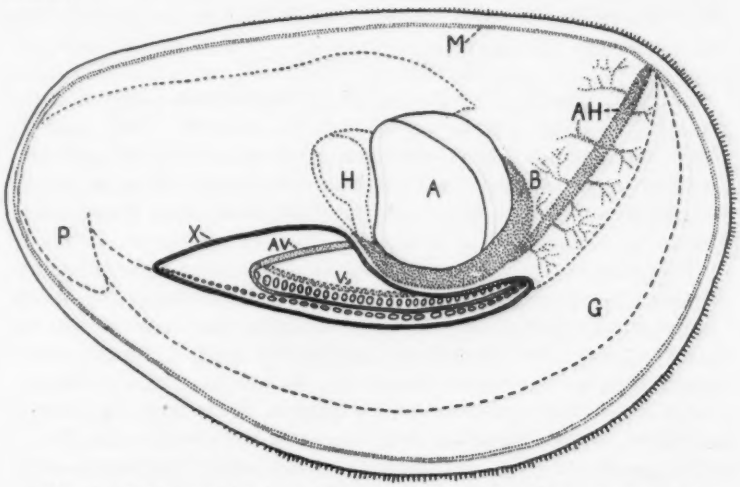


FIG. 1. Diagrammatic view of oyster from left side with part of mantle removed along double line (*X*). *A*, adductor muscle; *AH*, accessory heart (which is actually on inner face of mantle); *AV*, afferent vein; *B*, organ of Bojanus; *G*, outline of underlying gill; *H*, heart; *M*, marginal artery of mantle; *P*, labial palp; *V*, medial vein forming insertion of the two medial demibranchs.

Although not previously recognized as active organs, Kellogg (1890, Fig. 9) shows them in a figure of a cross-section through the cloaca, but refers to them only as ridges "running backward on inner wall of mantle, continuous with line of concrescence of gill to mantle." He failed to identify them as vessels since they were probably fixed in a state of contraction.

It proved difficult to inject the accessory hearts of a living specimen because of their marked sensitivity to mechanical stimulation. Even in an oyster anaesthetized with magnesium sulphate the slightest stimulation of the vessels caused them to contract. By leaving oysters in a

dilute solution of ammonium bromide in sea water for 24 hours, after which they were wide open and the adductor muscle completely relaxed, and then adding alcohol up to between 5 and 10 per cent, specimens were obtained in which all blood vessels, including both the heart and the accessory hearts, were completely expanded and insensitive to irritation. These were removed from the shell by cutting the adductor muscle close to its attachment without damaging the mantle. Injections of three kinds were made with suspensions of carmine or ink in sea water: into the ventricle; into the auricles, with the ventricle tied off; or into either of the accessory hearts. A fine cannula was used to inject the accessories so that the fluid could flow in both directions.

The Circulatory System

Complete description of the anatomy of the circulatory system is not necessary here, for various investigators (Leenhardt, 1926; Kellogg, 1890) have published such studies on *O. virginica* and *O. angulata*. However, it is necessary to describe the circulatory system in general in order to arrive at an understanding of the function of the accessory hearts.

When the ventricle is injected, the fluid enters the adductor muscle through the posterior aorta, and the viscera, labial palps, and mantle through the anterior aorta. The anterior aorta runs forward near the dorsal surface of the visceral mass, giving off along its course several large arteries to the visceral organs. At the anterior end it opens into a large vessel running dorso-ventrally adjacent to the hinge ligament of the valves. From this vessel there are five main branches, the first of which goes to the palps. At both dorsal and ventral extremities it divides into a pair of pallial arteries which run around the margin of the mantle. The ventral pallial artery of each lobe is continuous with the dorsal pallial artery of the same side through the marginal artery, which makes a complete circuit of the mantle lobe. The marginal artery sends small branches distally to the border of the mantle and tentacles, and large ones proximally into the tissues of the mantle proper.

Blood is collected in the lacunae of the mantle and carried directly into the auricles. When either the ventricle or the auricles are injected, the fluid enters neither the kidneys nor the accessory hearts. Because of the fact that the venous system consists of blood spaces which generally lack definite walls it is most difficult to trace the course of blood returning from the tissues. Apparently blood from the viscera, palps, gills and adductor muscle enters a large vein which forms the line of union of the two medial demibranchs. This vein is enclosed within the ventral wall of the visceral mass from the anterior end of

the gills to a point below the adductor muscle, posterior to which the gills hang free. Veins from the lateral demibranchs enter the medial vein by way of the septa which separate the gill lamellae into canals. On either side, about halfway between the adductor muscle and the anterior end, a vein runs from the corresponding gill into the lacunae of the excretory organ.

By injecting into one of the accessory hearts so that the fluid could proceed in both directions it was found that it entered not only the kidney but also the gills and the marginal arteries of both lobes of the mantle, as well as the other accessory. This was most surprising since the marginal arteries had been shown to receive direct arterial blood from the anterior aorta. It appeared to mean that the accessory hearts, by pumping blood from the kidneys into the marginal arteries, are forced to work against the arterial pressure already in the arteries.

That this is the case was shown by injecting carmine suspension into the ventricle until both marginal arteries, and branches from them into the mantle, were well penetrated. Then a suspension of blue ink was injected into one of the accessory hearts. Traveling distally the blue fluid entered the marginal arteries of both mantle lobes, crossing from one to the other through the connecting septum, and forced the red fluid back anteriorly. The blue replaced the red suspension in the arteries, and the branches from them into the mantle showed red in the finer subdivisions with blue in the trunks. Along its course each accessory heart gives off numerous small branches directly into the adjacent portions of the mantle.

Centrally the accessory hearts are continuous with the excretory organs, from which they receive blood. The organ of Bojanus is a rather indefinite structure within the mantle, extending laterally from the posterior surface of the adductor muscle around the ventral side of the muscle to a point below the pericardium. It consists of finely divided blood spaces in which the excretory tubules are suspended. A colored fluid forced anteriorly from the accessory heart passes with difficulty through this organ and into the veins or lacunae which collect blood from the viscera and adductor muscle. Some confusion was caused by the fact that in certain cases the fluid also penetrated the auricles when this injection was made, but it was found that this occurred only when the fluid was injected with considerable force.

It would appear that the accessory hearts play a very important rôle in completing the blood circulation through the excretory and respiratory organs. The mantle of lamellibranchs, with its rich supply of superficial blood spaces, is considered to be the primary organ of respiration, while the gills function chiefly to pump water from which they

extract food particles and transmit them to the mouth (Galtsoff, 1928; Nelson, 1923; Hopkins, 1933). The gills receive all of their blood, and the mantle at least a part of its supply from the excretory organ, and the blood from the latter is returned directly to the auricles.

Activity of the Accessory Hearts

In a large specimen the activity of the accessory hearts is most conveniently observed. Japanese oysters from 15 to 20 cm. long were found to have large, well-developed accessory hearts which could be readily studied. In such a specimen they may be observed as a pair of long vessels on the inner surface of the mantle, within the cloacal chamber, which when contracted are not more than 1 mm. in diameter, but which expand, as they fill with blood, to a diameter as great as 5 mm. They may be visible for a length of 2 to 4 cm. so that waves of contraction are easily seen. When a specimen is opened, by cutting the adductor muscle, the accessory hearts, like the heart itself, do not function normally but waves of contraction run in all directions. If the specimen be placed in running sea water for a few hours they begin to pulsate in an orderly manner.

In order to observe with certainty the normal activity of these organs, however, a method was employed whereby it was not necessary to cut the adductor muscle or otherwise injure the specimen. Portions of the right, or upper, valve were carefully sawed away in such a manner as to avoid injuring any underlying tissue. The free parts of the right mantle lobe characteristically would fold back over the cut edge of the shell, exposing the mantle chamber and cloaca. Through the latter chamber the pericardium could be seen and the rate of pulsation of the heart observed while at the same time the accessory hearts were exposed to view. Studies on such an oyster should result in an understanding of the normal activity of the organs, for the circulatory system was completely intact.

It was not feasible to record the pulsations of the accessory hearts by the kymograph method, for they are extremely sensitive. A pulsation begins, when the organ is fully expanded, as a constriction at the central end, next to the kidney, and extends slowly outward. The direction of the wave is very distinct. If the distal end be stimulated mechanically at the time a wave of contraction is starting at the proximal end the result, for several minutes, is a confusion of ineffective waves of contraction passing in both directions. An unsuccessful attempt was made to observe blood cells within the accessory hearts so as to be sure that the blood flow is from the kidney to the mantle.

In order to determine whether a definite relationship exists between the rate of heart-beat and the rates of pulsation of the accessory hearts, counts were made consecutively of the time required for a given number of pulsations by each. In Table I a typical series of such measurements is given, showing that the three organs need not beat at the same rate. The accessories pulsate at a rate only one-third to one-fourth that of the heart, and the two accessories do not act either in unison or at the same rate.

TABLE I

Comparative rates of pulsation of the heart and the two accessory hearts

Time	Temperature	No. Pulsations per Minute		
		Heart	Right accessory	Left accessory
	°C.			
9:25	17.2	15.7	2.5	6.3
9:29	17.3	16.1	4.5	6.2
9:33	17.4	16.8	5.0	7.1
9:57	18.1	17.9	4.5	8.0
10:01	18.2	17.1	5.1	7.1
10:05	18.3	17.7	4.8	8.0
10:46	19.4	20.1	5.3	8.0
11:20	19.8	21.3	6.4	8.6
2:34	19.9	18.5	7.6	6.6
2:38	19.9	16.5	6.5	6.5
3:03	18.8	17.9	6.6	7.6
3:06	18.8	18.6	6.9	7.2
3:56	18.5	16.8	6.1	9.0
3:59	18.5	17.3	5.5	6.4
4:20	18.5	18.2	9.9	9.1
4:23	18.5	18.2	8.9	9.1
Average		17.8	6.0	7.5

The results suggest that the rate of pulsation of the accessories is determined only by the rate at which they fill with blood, and that in this respect only is their activity dependent upon that of the heart. In further support of this assumption is the fact that typically the left accessory pulsates more rapidly than the right, which may be due to the oyster's position upon its left, or cupped valve, so that gravity causes the lower, or left, accessory to fill with blood more rapidly.

COURSE OF CIRCULATION

The circulatory system of the oyster, as has been stated above, must be looked upon as consisting of two incompletely separated parts (Figs. 1 and 2). The heart pumps blood through the arteries to the adductor

muscle, labial palps, and viscera, from the lacunae of which it is collected and carried to the excretory organs. The heart also pumps blood directly into the marginal arteries of the mantle lobes. From the excretory organs the accessory hearts force the blood into the gills and into the marginal arteries, which therefore receive both arterial and venous blood. It is remarkable that such large vessels as the marginal arteries should receive both types of blood and, since the lacunae of the mantle carry blood directly back to the auricles, the heart receives a mixture of blood only a part of which has passed through the excretory organs. A marginal artery is a complete loop, into one side of which the heart pumps arterial blood, while the accessory hearts pump venous blood into it at the opposite side. The loop, then, is subjected, along its course through the mantle, to arterial pressure from both directions as well as to venous pressure from the accessory hearts. This pressure presumably forces the mixed arterial and venous, purified and unpurified, blood through the smaller vessels of the mantle, assuring aëration by means of the current of water produced by the gills.

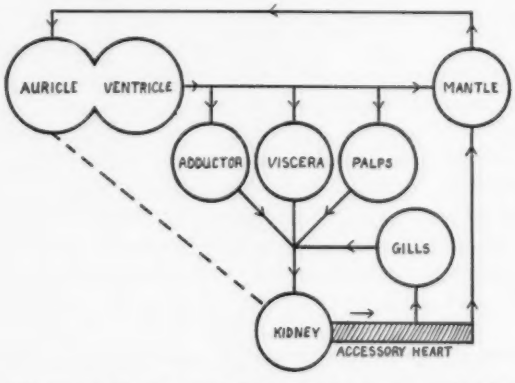


FIG. 2. Diagram of the course of circulation in the oyster showing part played by accessory hearts.

A schematic diagram of the apparent course of circulation is presented in Fig. 2. Because of the difficulty of tracing the veins in the vicinity of the excretory organs it is uncertain whether or not the blood spaces of these organs are actually continuous with the auricles. On the other hand, the veins from the mantle may be traced directly into the auricles.

This interpretation differs in some respects from the description of *Gryphea* (*Ostrea*) *angulata* given by Leenhardt (1926, p. 82): "Le

sang provenant de la masse viscérale passe tout entier par l'organe excréteur. Il n'en est pas de même du sang revenant des palpes labiaux qui se déverse directement dans les vaisseaux ramenant le sang à l'oreillette; de même le sang revenant du manteau est en grande partie déversé directement dans les veines afférentes du cœur." Kellogg (1890, p. 408) described the lamellibranch venous circulation as follows: "From the irregular sinuses into which the arteries empty, the blood is collected in larger vessels and conveyed to a vessel beneath the pericardium, called the sinus venosus. Thence it passes to the gills, traversing on the way the walls of the nephridia, where waste products are excreted. The circulation is completed by the return of the blood from the gills to the auricles of the heart."

Apparently veins from the visceral mass, palps, and muscle open into the large medial sinus (Fig. 1, *V*) which forms the insertion of the two medial demibranchs. By injecting a colored fluid into this sinus the small vessels of the gill filaments are penetrated, showing that the efferent blood from the gills is not kept apart from the rest of the venous blood. This vein communicates with the longitudinal vein of each gill through the connectives separating the gill into canals. From each longitudinal vein (Fig. 1, *A.V.*) a large vein runs to the venous sinus which communicates directly with the excretory organ. The sinus may also open into the auricles, though it is difficult to be sure. On the other hand, the veins returning blood from the mantle to the auricles are well defined and may readily be traced.

The marginal vessels of the oyster appear to be entirely different from those of the mussel, as described by Field (1922) and others, and the course of the circulation in the two cases is decidedly different. According to Field, the aorta of the mussel runs anteriorly along the dorsal surface of the viscera, giving off branches to the mantle lobes. Around the border of the mantle runs the marginal sinus, which collects blood from the mantle and carries it through a large vein to the kidney, from which it passes directly into the heart. The mantle then receives only arterial blood. With the blood spaces of the kidneys communicating directly with the auricles by a large vessel, it may be considered that accessory hearts are not necessary, that without such assistance the circulation of the blood is completed.

It may be that with respect to the accessory hearts oysters represent an exception among lamellibranchs, though it would be of interest to determine definitely whether this is the case. Grave's (1909) description of the wing-shell, *Atrina,* suggests that accessory hearts may be found in this species also. He stated (p. 427), "After bathing the glandular cells of the kidney the blood is collected into a large vein

which transports it to the gills to be aërated." He apparently did not observe pulsations of this vein, though such an observation would be of interest.

Among mollusks it would seem that the only structures known which have a function comparable to the accessory hearts of the oyster are the gill hearts of cephalopods. These organs have long been known as remarkable examples of pulsating blood vessels. Their function is to pump blood from the excretory organ, where it is collected after passing through the viscera, to the gills, from which it returns to the heart. The accessory hearts of the oyster appear to be homologous to the cephaloped gill hearts in that they take origin in the vessels of the kidneys and pump blood to the respiratory organs, in this case primarily the mantle and secondarily the gills. In both groups the function is the same; namely, to return venous blood from the excretory organ to the auricles by way of the organs of respiration. The term, *gill hearts,* might also be used to refer to these structures in oysters.

Although all of the observations described above were made on *Ostrea gigas,* it was determined that both *O. virginica* and *O. lurida* possess similar pulsating accessory hearts. The structures appear to be characteristic of the genus.

Summary

In three species of *Ostrea* a pair of actively pulsating accessory hearts was observed in the lateral walls of the cloacal chamber. They appear to pump blood from the lacunae of the excretory organs into the large marginal arteries of the mantle lobes and into the gills.

Counts of the rate of pulsation of heart and accessory hearts in *O. gigas* show that they act independently, the rate of pulsation of the accessories probably being determined by the rate at which they fill with blood. Only in this respect is their rate dependent upon the heart rate.

The accessory hearts appear to be homologous to the gill hearts of cephalopods.

The course of circulation, as influenced by the accessory hearts, is apparently as follows: blood from the ventricle passes through large arteries to the viscera, adductor muscle, and palps; the anterior aorta is directly continuous with the marginal arteries of the mantle; venous blood from viscera, palps, gills, and muscle passes to the excretory organs from which it is pumped by the accessory hearts into the marginal arteries of the mantle and to the gills; blood collected from the mantle returns directly to the auricles.

Heart and accessories both pump blood into the marginal arteries, which therefore receive both venous and arterial, purified and unpurified blood.

LITERATURE CITED

VON BUDDENBROCK, W., 1928. Grundrisz der vergleichende Physiologie. Berlin.

FIELD, I. A., 1922. Biology and Economic Value of the Sea Mussel, Mytilus edulis. *Bull. U. S. Bur. Fish.*, **38**: 127.

GALTSOFF, PAUL S., 1928. Experimental Study of the Function of the Oyster Gills and its Bearing on the Problems of Oyster Culture and Sanitary Control of the Oyster Industry. *Bull. U. S. Bur. Fish.*, **44**: 1.

GRAVE, B. H., 1909. Anatomy and Physiology of the Wing-shell Atrina rigida. *Bull. U. S. Bur. Fish.*, **29**: 409.

HOPKINS, A. E., 1933. Experiments on the Feeding Behavior of the Oyster, Ostrea gigas. *Jour. Exper. Zool.*, **64**: 469.

KELLOGG, J. L., 1890. A Contribution to our Knowledge of the Morphology of Lamellibranchiate Mollusks. *Bull. U. S. Bur. Fish.*, **10**: 389.

LEENHARDT, H., 1926. Quelques études sur Gryphea angulata (Huitre du Portugal). *Annales de L'institut Océanographique,* Nouvelle Série, **3**: 1.

NELSON, T. C., 1923. The Mechanism of Feeding in the Oyster. *Proc. Soc. Exper. Biol. and Med.*, **21**: 166.

MODIFICATION OF BEHAVIOR IN NEREIS VIRENS

MANTON COPELAND AND FRANK A. BROWN, JR.

(From the Searles Biological Laboratory, Bowdoin College, Brunswick, Me.)

In a recent paper (Copeland, 1930) the senior writer described an experiment on *Nereis virens* which indicated that the worm formed a conditioned response to both increased and decreased illumination. The results were made possible through the fact that *Nereis* will remain in a glass tube placed in sea water, and there behave essentially as it does in its natural burrow in the sand.

Under laboratory conditions one of its most interesting reactions is that to food (Copeland and Wieman, 1924). If, for example, a fragment of clam is dropped or held at the end of a tube occupied by a worm carrying on its characteristic undulatory movements of the body (Fig. 1, *A*), juices from the clam are drawn into the tube which stimulate the animal. Its response is a forward movement through the tube continued, often rapidly, until it arrives at the end (Fig. 1, *B* and *C*), when the food is seized. If the observer desires he may feed the worm by holding the piece of clam in forceps as illustrated in Fig. 1, *H*, *I*, and *J*. When, however, the food is removed after stimulation occurs the worm, on coming to the end of the tube, in most instances extends the anterior portion of the body outward, swinging it to the right or left or to the surface of the water in an attempt to find the clam. If the animal is particularly positive to the food stimulus it may stretch far outward and carry on active swaying and turning movements for a period of several minutes before withdrawing into the tube. These searching movements, as we shall designate them, are extremely characteristic of *Nereis* under food stimulation, and are readily recognized in various degrees of intensity by one who is at all familiar with the behavior of the species. Some of the positions assumed by a worm at this time are shown in Fig. 1, *D*, *E*, *F*, and *G*. It is this remarkably active and definite reaction of *Nereis* to food substances that gives the experimenter a favorable opportunity to test its ability to form a conditioned response. Having studied the worm's behavior when a suddenly modified illumination was used to signify the presence of food, it appeared of interest to try in a similar way a tactile stimulus to which the animal usually is more negative than positive.

Three worms, collected October 14, which will be designated *A*, *B*,

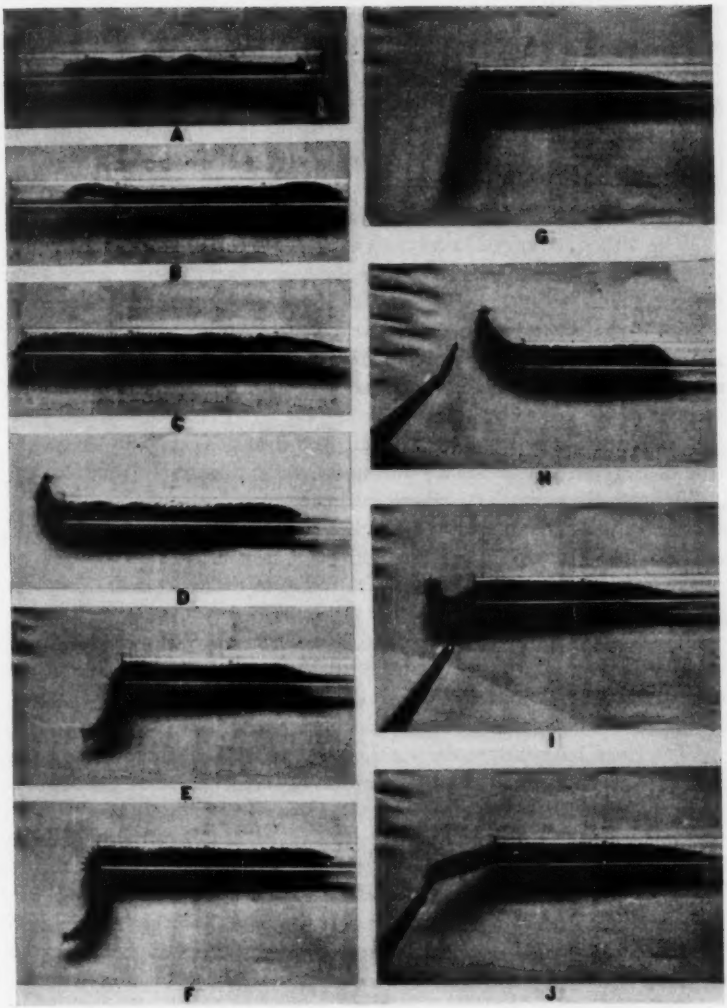


FIG. 1.

A. Nereis virens in a glass tube carrying on undulatory movements of the body which draw the sea water through the tube.

B. Worm moving forward after being stimulated by materials emanating from a fragment of clam held for a few moments at the end of the tube.

C. Worm starting to advance beyond the mouth of the tube.

D, E, F, G. The anterior end of the body, extended several centimeters out of the tube, is moved in various directions as the worm attempts to find the food which has been taken away. The animal is now exhibiting typical searching movements.

H, I. A piece of clam, held in forceps, is being offered to the worm.

J. Worm taking the clam from the forceps.

and *C*, were selected for the experiment. On being brought into the laboratory they occupied glass tubes which were placed in shallow square dishes of Pyrex glass, measuring about 21 cm. across the bottom and 3 cm. in height, containing sea water to the depth of about 2.25 cm. The tubes, approximately 10 cm. in length, with inside diameters of slightly over 5 mm., were large enough to allow the worms to carry on undulatory movements of the body and to turn around without difficulty.

The dishes, arranged in a row 8 cm. apart, were placed on a support painted white so that they paralleled the base of an east window 88 cm. distant. The buff-colored shades of this window, and another on the same side of the room, usually were pulled down to within 12 cm. of the bottom to protect the worms from the direct rays of the morning sun. Since there were no other windows in the room, the experimenter could carry on his work without shading the animals.

A small stone resting on each tube held it in position 6 cm. from the side of the dish nearer the window and parallel to it.

Beneath the dishes and tubes centimeter scales were marked off, and at both ends of the tubes three concentric circles were traced with radii of 1, 2, and 3 cm. By this method of graduation it was possible to record at any time the position of the worm in the tube, or its progress outward.

In order to keep the physiological state of the animals as near normal as possible, the sea water was changed about every four days and the dishes carefully cleaned in tap water. At no time was a worm tested within four hours after the water had been renewed.

The room temperature during the experiment varied between 14° C. and 28° C., but no resultant modification in the behavior of the animals was noted.

On October 24 ten preliminary tests were begun to determine the reactions of the worms to a tactile stimulus. To effect this stimulation a delicate, blunt-ended glass rod was used. It was bent at a right angle about 2.5 cm. from the end in order that it might easily be inserted into the tube containing the worm. Great care was taken to keep the rod perfectly clean. The end with which the worm was touched was not handled, and before and after each test it was washed in distilled water and dried with filter paper. If the worm was completely inside the tube the rod was carefully inserted and the animal's tentacular cirri, or other portions of the head region, were lightly touched. Immediately afterward the rod was withdrawn and the behavior of the worm recorded over a period of two minutes.

In order to avoid the possibility of the worms forming an association between touch and food in the preliminary trials, they were fed and

given tactile stimulation on alternate days. At every feeding they received four pieces of clam 2 or 3 mm. in diameter. These were always placed at that opening of the tube toward which the animal's posterior end was directed. After the worm turned about it usually began wave-like movements of the body drawing sea water and juices from the clam into the tube. By this method, therefore, the worm was stimulated chemically in most, if not in all, cases before there was any contact with the clam whatsoever.

On the days when the worms were touched with the glass rod they were tested usually twice, once in the morning and again in the afternoon. Occasionally, however, there was but one trial during the day.

The reactions to the rod were all graded from 10, the most positive, to 0, the most negative. These eleven types of response may be briefly described as follows:

10. Worm moves forward at once, extending its anterior end out of the tube. The head is moved about in various directions, just as it is when the animal is chemically stimulated by the juices of clam and is searching for food, a striking type of response already described. It continues for two minutes, and during the period the head is 2 cm. or more out of the tube.

9. Worm advances immediately and exhibits searching movements as in the preceding case. At some time it extends out of the tube 2 cm. or more, but at another time *draws back* into the tube, or to the end of it.

8. Worm moves forward beyond the mouth of the tube at once and shows searching movements as in 10 and 9. At all times during the 2-minute period, however, it is *less* than 2 cm. out of the tube, and it may during the time withdraw to the end of the tube, or into it.

7. Worm withdraws farther into tube. Before 2 minutes have passed, nevertheless, it moves forward to the end of the tube, or beyond the opening.

6. Worm attempts to grasp the end of the glass rod, or it may stretch forward several millimeters toward it, but afterwards it is motionless to the end of the period. No searching movements occur.

5. There is no visible reaction, positive nor negative.

4. Worm withdraws, but in less than 2 minutes moves forward. It does not progress, however, so far as the end of the tube.

3. Worm withdraws, but starts undulatory movements of the body (Fig. 1, *A*). By this activity not only is respiration aided but the animal's chemical environment is tested. If these movements are going on when the worm is touched they are continued.

2. Worm withdraws less than one centimeter and then *remains quiet*. Undulatory movements are *not* started.

1. Worm withdraws *more* than one centimeter, but *less* than two, and remains quiet.

0. Worm withdraws *more* than two centimeters and shows no further movement.

Although the difference between any two successive grades of reaction as outlined above is not great, forms of response which vary by two or three points are decidedly distinct, particularly to one familiar with the feeding behavior of *Nereis*.

On the completion of ten preliminary tests the results recorded for each worm were averaged and the first points of the three curves determined (Fig. 2, points I). The animal with the lowest average, or the one most negative to touch, was selected for immediate training. This proved to be worm *A*.

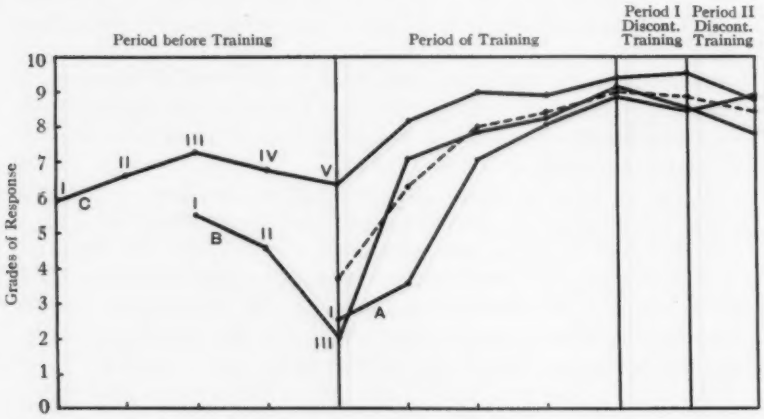


FIG. 2. Curves showing the changes in the reactions of worms to tactile stimuli alone (points I–V) and when followed by feeding (period of training). Their responses after two periods of interrupted training are indicated on the right. Each point on the curves represents an average of ten successive trials, and the dotted line shows the average of the three curves.

The procedure noted above was continued with worms *B* and *C*, the former for twenty more tests (determining points II and III of Fig. 2), and the latter for forty more (determining points II, III, IV, and V of Fig. 2), before their training periods were begun. During this time, therefore, these two worms served as controls. The same method of grading responses to tactile stimulation was used in the experiments to be described.

Period of Training

The training of worm *A* began on November 10, or two days after the completion of the first ten trials. During the period the same glass rod was employed in touching the worm and its care and manner of use were in no way modified. Again the behavior of the animal was noted for 2 minutes following tactile stimulation. At the end of that time, however, the worm *was fed*. This was accomplished by placing a small fragment of clam on the tip of another glass rod of approximately the same size and shape as the first. When the food was presented to the worm it was quickly seized and removed. If it happened that the head of the animal was inside the tube the end of the rod holding the clam was slowly inserted into the tube, when almost invariably juices emanating from the food stimulated the worm to move forward and take it. After using the rod it was washed in tap water and dried.

The worm was touched at least once a day, and usually twice, and was fed one piece of clam after each test. Thus the amount of food given it was about equal to that received before the training began and approximately the same as that taken by the controls. At the end of every ten trials an average grade of response was obtained which determined the points on the curve shown in Fig. 2.

After the completion of thirty[1] preliminary tests the training of worm *B* was started, worm *C* still being used as a control. It was not until December 26, when it had been given fifty tests with the glass rod unaccompanied by feeding that the training of worm *C* began. The same rods were used and the technique was unmodified.

The training periods of worms *A, B,* and *C* were closed November 30, December 23, and January 23 respectively, after each worm had been tested forty times.

The modification of the response to contact stimuli is shown by the curves in Fig. 2. From the beginning to the end of the training period there is a rise in the grades of the three worms varying from 3.0 in the case of worm *C* to 7.1 for worm *B*. On the other hand, before this period began the average of the last ten trials on worm *C* (Fig. 2, point V) shows a rise of only 0.5 when compared with the average of the first ten (Fig. 2, point I), whereas in the case of worm *B,* which was given thirty trials before training started, the third point on the curve (Fig. 2, point III) indicates a drop of 3.4.

Attention may be called to individual differences in the responses of the three worms to tactile stimulation. The averages of the first ten tests (Fig. 2, points I) led to the conclusion that worm *C* was least negative and worm *A* most so, and this order was still maintained at the close

[1] Through error thirty-one tests were made. The last one was disregarded.

of the training periods. During this time, however, the three learning curves gradually converged until the range of variation in response was reduced from 3.3 grades to 0.5. It appears, therefore, that the differences in the reactions to touch existing at the start of the experiment were caused nearly, though not quite, to disappear.

First Period of Discontinued Training

Having definitely modified the behavior of the three worms, it became of interest to determine how far the induced changes would be sustained over a period during which stimulation with the glass rod was discontinued. Accordingly, between the dates which marked the close of the preceding period and February 14, the worms were given four pieces of clam on alternate days. No independent tactile stimulation preceded the offering of food which was taken from the end of a glass rod. Contact occurred, therefore, only when the clam gently touched the head or mouth parts, in all probability about simultaneously with chemical stimulation. By February 14 worm *A* had been treated in this way for 75, worm *B* for 52, and worm *C* for 21 days.

Table I

Table showing grades of response of the three worms to tactile stimulation in successive trials at the beginning and end of the experiment

Worm	First Ten Trials *			Last Ten Trials of Training Period			Ten Trials at Close of First Period Discontinued Training			Ten Trials at Close of Second Period Discontinued Training		
	A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
Trial 1.....	0	2	1	7	8	10	7	7	7	10	7	7
" 2.....	1	6	6	10	8	9	4	10	10	10	7	8
" 3.....	3	10	6	10	10	9	8	10	10	10	9	8
" 4.....	0	5	10	10	10	10	7	8	10	10	8	10
" 5.....	4	4	5	8	10	10	10	8	9	8	9	9
" 6.....	6	0	6	10	10	9	10	6	10	8	7	9
" 7.....	2	6	6	4	10	10	10	10	10	9	8	9
" 8.....	3	6	5	10	8	10	10	8	10	8	8	10
" 9.....	2	8	6	10	8	9	10	9	10	8	7	9
" 10.....	5	8	8	10	10	8	9	10	10	8	8	9
Average	2.6	5.5	5.9	8.9	9.2	9.4	8.5	8.6	9.6	8.9	7.8	8.8

* The averages determined points I of graph.

In order to determine how much the modified behavior of the worms had changed during these intervals they were given ten tests by the method used in training them; namely, by first touching the animal with the rod and, at the end of 2 minutes, feeding it. The trials begun on February 14 were concluded February 20, when the results for each animal were averaged and the points plotted on the graph (Fig. 2).

It will be noted that the curves of two of the worms dropped only slightly; that of worm *B* 0.6 grades after 52 days without training, and worm *A* 0.4 grades after 75 days. On the other hand, the curve of worm *C*, after only 21 days without training, rose 0.2. An examination of Table I shows that in the case of worm *C* the average of the first five of the ten trials gave a response grade of 9.2 which is not above that (9.4) recorded at the close of the long period of training. The average of the last five tests, however, was 10. It appears, therefore, that this worm modified its behavior in the short series of ten trials, in truth, after the first, more than enough to offset any loss from 21 days lack of experience.

Second Period of Discontinued Training

After the trials reported above were completed the worms were not given further training for 35 days, although again they were fed four pieces of clam on alternate days. The manner of feeding, however, was modified in order to eliminate as far as possible any tactile stimulation. Instead of offering the clam from the end of a glass rod, by which method possibly the worm was occasionally touched by the food before it was recognized as such, the fragments were placed in the dish 2 or 3 cm. from that end of the tube toward which the posterior extremity of the animal was directed. Later, after turning about in the tube, and in most instances carrying on undulatory movements of the body, the worm received at first only chemical stimulation from the clam. It then advanced and seized the fragments.

The change in the worms' behavior at the close of this period was determined as before by giving them ten tests in which tactile stimulation by means of the glass rod was followed by feeding. The trials were begun March 26 and completed April 3, and the averaged reaction grades for each worm are indicated by the last points on the graph (Fig. 2).

As a result of this modification in the manner of feeding a sharper decline in the curves perhaps was to be expected, and the one representing the average of the three individuals does show this. Worm *A*, however, proved to be slightly more positive to tactile stimulation than it was before the training was discontinued for 35 days. It should be noted, nevertheless, that the curve of this worm is at no time higher than it was at the close of the training period.

A study of the table showing the results of individual tests on the three worms at the beginning and end of the experiment reveals not only a marked change from more negative to more positive reactions to tactile stimuli when associated with food, but also the persistence of this modified behavior. None of the forms of response below type

seven involves food-searching movements (Fig. 1, *D*, *E*, *F*, and *G*), and twenty-five of the first thirty tests (ten on each individual) show an absence of such movements. At the end of the training period, however, only one test in thirty is graded below seven; in fact, only two below eight. At the close of the first period of interrupted training two tests of the thirty grade below seven, and at the end of the second period none at all. In view of the fact that the worms experienced no contact with the rod for over a month before the final thirty tests were made, the results indicate a decided retention of modified behavior.

Summary and Conclusions

1. *Nereis virens* when touched at the anterior end with a glass rod usually showed a reaction which was more negative than positive in character, whereas the response to chemical stimulation by materials derived from clams was decidedly positive.

2. When the tactile stimulus was followed by the presentation of fragments of clam three worms soon appeared to associate the two and learned to respond to touch in a more positive way. Finally after forty trials (for the most part given twice a day) the behavior of the worms following tactile stimulation alone could not be distinguished from that of worms which had been chemically stimulated by clam juices and were searching for food.

3. After periods of discontinued training the average of the three learning curves dropped slightly.

4. The results of this experiment are in accord with those of a previous one to which reference has been made (Copeland, 1930), where change in illumination was used as a sign for food, and indicate that *Nereis* readily forms a conditioned response.

References

Copeland, Manton, and H. L. Wieman, 1924. The Chemical Sense and Feeding Behavior of Nereis virens, Sars. *Biol. Bull.*, **47**: 231.

Copeland, Manton, 1930. An Apparent Conditioned Response in Nereis virens. *Jour. Compar. Psych.*, **10**: 339.

THE CHEMICAL NATURE OF THE PIGMENTS AND THE TRANSFORMATIONS RESPONSIBLE FOR COLOR CHANGES IN PALÆMONETES

F. A. BROWN, JR.

(From the Biological Laboratories, Harvard University, and the Woods Hole Oceanographic Institution, Woods Hole, Mass.[1]*)*

I. The Chemical Nature of the Pigments

The most prevalent pigment of *Palæmonetes vulgaris* is a reddish one which in the living shrimp varies in tint from a clear red to a brownish black, and in consistency from a homogeneous, viscous liquid to a granular gel. The yellow pigment, like the red, is always located within definite chromatophores. This pigment is a transparent, homogeneous liquid less viscous than the red. The yellow and red pigments, though most commonly present in the same chromatophore center, always retain their individuality as two distinct masses of pigment.

Lönnberg and Hellström (1932) studied spectroscopically the two pigments that they found in the lobster. In distribution between petroleum ether and methyl alcohol a yellow pigment remained in the petroleum ether (absorption bands in petroleum ether, 490 and 457 mμ) and a reddish pigment went into the alcohol layer (absorption band in ether, 494 mμ). Kuhn and Lederer (1933) have worked upon the brownish black pigment of the Norwegian lobster, *Astacus gammarus,* and have determined it to have the chemical formula $C_{27}H_{32}O_3$ and to this they gave the name astacin. The absorption band of astacin in pyridin was found to be very broad and to have a maximum at 500 mμ. These men also found in their extracts a small amount of a substance which they admitted was probably carotin. Later, Fabre and Lederer (1934) showed that astacin was present in a number of other crustaceans. Very recently Karrer and Loewe (1934) have concluded that astacin was a derivative of β carotin.

A study has been made of the absorption spectra of the pigments of *Palæmonetes.* Shrimps were boiled to convert the blue pigment to red, then dried and extracted with chloroform. The resulting extract was orange-red in color. It was quite photosensitive, bleaching to a pale yellow during a few hours exposure to sunlight. Figure 1 shows the absorption spectrum of the pigment freshly extracted in chloroform. There is a single asymmetrical band with a maximum at about 480 mμ.

[1] Contribution No. 55.

This band is roughly the same shape as that determined by Redfield (1930), who examined a chloroform extract of the blood of the lobster, *Homarus*. The absorption spectrum of the lobster blood extract is also shown in Fig. 1.

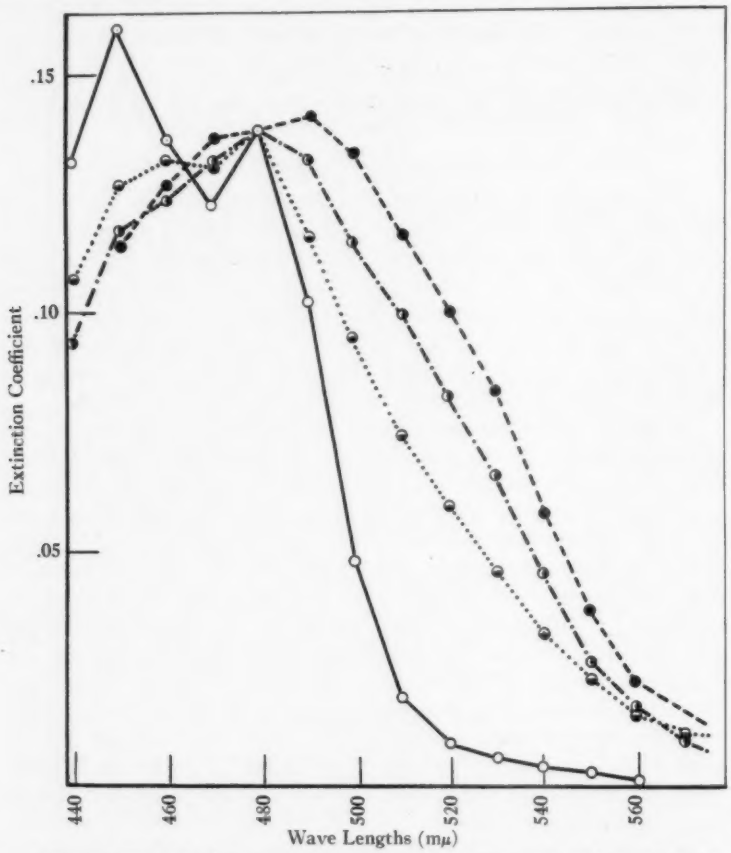


FIG. 1. The unbroken curve shows absorption spectrum of shrimp yellow pigment; – – – – shows absorption band of extract of lobster blood (Redfield, 1930); ····· shows absorption spectrum of extract of shrimps which have little red pigment in chromatophores; and —·—·— shows the absorption band of extract of shrimp which have much red. All measurements are made of pigments in chloroform solution.

Kuhn and Lederer have found that when a petroleum ether solution of astacin is shaken with a solution of sodium hydroxide in 90 per cent

methyl alcohol the astacin enters the methyl alcohol layer as a sodium salt of astacin. When this layer is acidified the astacin comes out of solution as violet crystals. It has been found that if the pigment obtained by extraction of the whole *Palæmonetes* with chloroform was

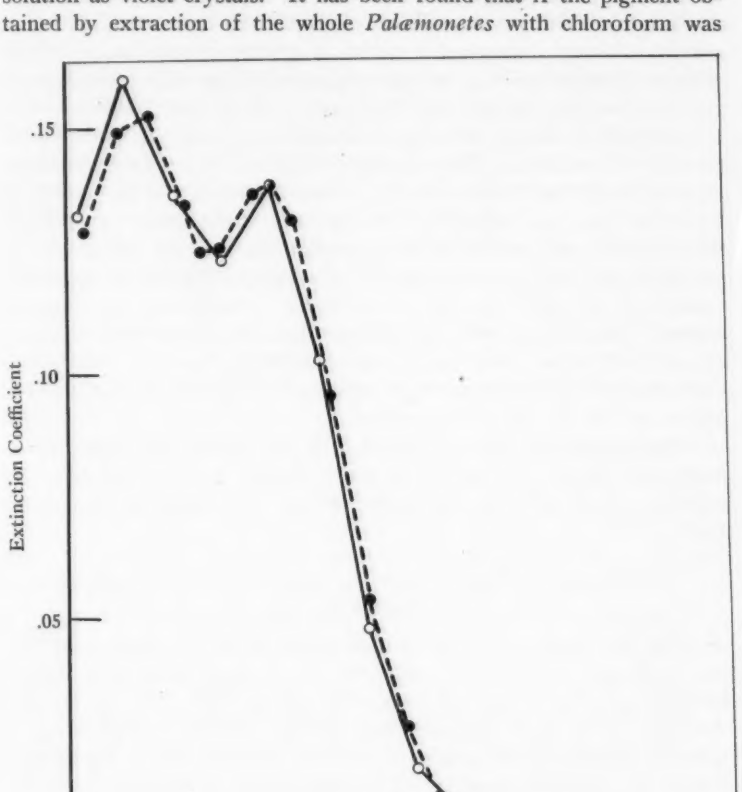


FIG. 2. The unbroken line represents the absorption bands of shrimp yellow pigment in chloroform; – – – – shows the absorption bands of plant carotin in alcohol and ether as measured by McNicholas (1931).

dissolved in petroleum ether and then shaken with a solution of potassium hydroxide in 90 per cent methyl alcohol, a reddish pigment entered the methyl alcohol layer and a yellow pigment remained in the petroleum ether. Figure 2 shows the absorption spectrum of the petro-

leum ether component after it had been redissolved in chloroform. Plotted upon the same coördinates is the absorption spectrum of a solution of plant carotin in alcohol and ether as it was measured by McNicholas (1931). The shrimp pigment strikingly resembles plant carotin. Living *Palæmonetes* contain a greater proportion of yellow pigment than do lobsters and correspondingly a greater percentage of this fraction was obtained than was found by Kuhn and Lederer.

Samples of shrimp differing from one another in the relative proportions of red and yellow pigments visible in their chromatophores showed absorption spectra differing from one another in the extent to which the band was shifted in one direction or the other. The limits of this shift were defined by the absorption spectrum of carotin on the one hand and that of the lobster red pigment on the other. Figure 1 shows the absorption spectra of the whole pigment from two lots of shrimp. In one lot much red pigment was present in the chromatophores, and in the other there was considerably less. It thus seems probable that these differences are explained by the varying percentages of astacin and carotin in the extracts.

These results strongly suggest that the pigment of the yellow chromatophores of *Palæmonetes vulgaris* is carotin, $C_{40}H_{56}$, and that the pigment of the red chromatophores is one or another compound of astacin.

II. Chemical and Morphological Transformations Responsible for Color Changes

Thus far, nearly all the work upon color changes in crustaceans has been done upon the rapid responses of the chromatophore as indicated by the migration of pigment within the pigment cells. In reality, the well-adapted shrimps and prawns in nature owe their adaptations at least as much to the differential formation and destruction of pigments within the chromatophores as to the more rapid migration of the already formed pigments. Although this fact has been noted by the majority of workers in the field, little actual research has been devoted to it.

The following experiments were carried out upon adult *Palæmonetes vulgaris,* and were designed primarily to show the rate at which the pigmentation of the shrimps could be changed as a result of light, darkness, and black and white backgrounds.

Observations on Destruction of Red and Yellow Pigments

A shrimp was taken from a black background and placed upon white. In 20 minutes the red and yellow pigments had concentrated

considerably. A blue network was left behind in the region that was formerly covered by the red and yellow pigments. The concentrated yellow was a clear and homogeneous pigment but the red contained much of the blue pigment mixed with it, making it purplish red and chocolate in color.

In about six hours the blue network had gradually faded and the yellow and brownish pigments had completely concentrated.

For the following six or eight days there were faint bluish processes extending from the brownish pigment. The blue pigment seemed to be diffusing away from this chromatophore and at the same time the chromatophore was becoming more and more reddish.

At the end of eleven days there was practically no blue pigment left in the red, but clustered around the red were many small blue triangular crystals.

After fifteen days the red pigment had become homogeneous and had decreased to only a small fraction of its original amount. Globules of red pigment had left the chromatophore and were in the tissues around it.

At the end of three weeks nothing was left of the red pigment except small droplets scattered about. The yellow chromatophore was still intact and functional but had lost a large quantity of its pigment. Observations were continued for two more weeks. The yellow pigment continued to decrease in amount but was still present at the end of this time.

Plate I, Fig. 7, shows a red, yellow, and blue chromatophore after two days upon a white background, and Fig. 8 shows the same region of the same shrimp after sixteen days upon the same background. Notice the small droplets of red pigment that still persist. The time required for the complete disappearance of the red and blue pigments is a function of the quantities present at the beginning of the experiment.

Observations on Formation of Red and Yellow Pigments

An experiment upon formation of pigment was begun by selecting a shrimp which had been kept upon a white background for five weeks and then seeking a chromatophore region where only yellow pigment remained. A photomicrograph (Fig. 9) was taken of the region and then the shrimp was placed in a salt water circulating apparatus upon a black background. At the end of five days the region was again examined. The yellow pigment was broadly dispersed and had assumed a pale orange tint. Some new red pigment had commenced to form. This last was surrounded by the characteristic blue network. The shrimp was placed upon a white background and after an hour the

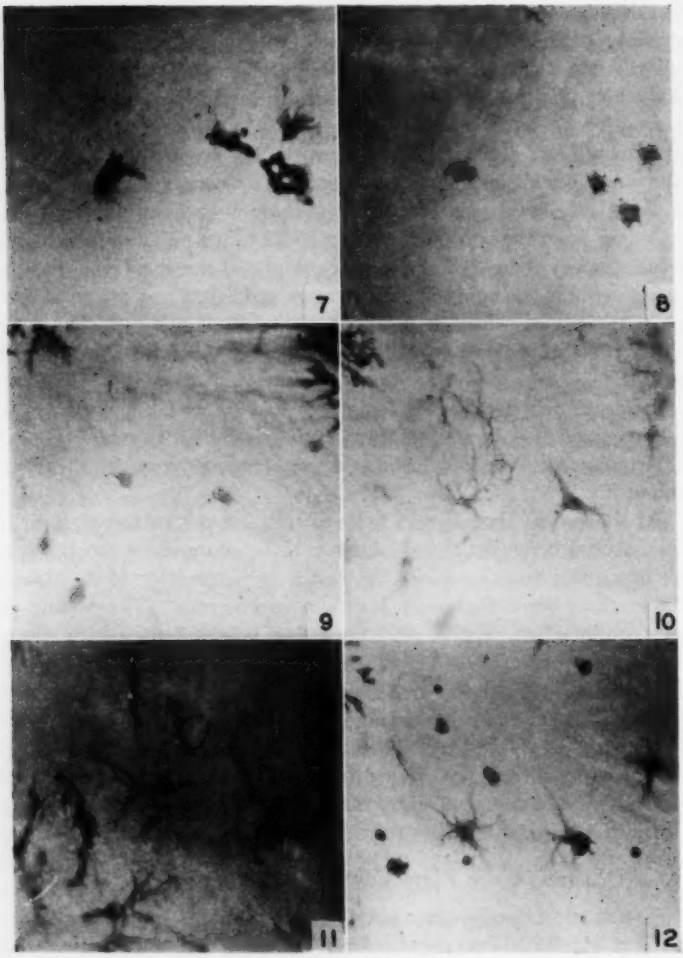


PLATE I

FIG. 7. Red and yellow chromatophores of a shrimp which has been two days upon a white background.

FIG. 8. Same area as in the preceding figure but photographed after two weeks upon a white background.

FIG. 9. Some chromatophores in a shrimp that has been five weeks upon a white background. Only yellow pigment is present.

FIG. 10. Same area as in Fig. 9, and showing the formation of red pigment after five days upon a black background.

FIG. 11. Same area as in Fig. 10, but photographed after two weeks upon a black background. Much red pigment has formed and a blue network is visible.

FIG. 12. Same area as in Fig. 11, but the animal has been one day upon a white background.

region was again photographed (Fig. 10). After two weeks upon a black background the shrimp was becoming quite dark in color. Much red had formed and the blue network was heavy. After an hour upon white the chromatophores of the experimental region were photographed (Fig. 11). This was repeated after 24 hours upon white (Fig. 12) in order to determine definitely whether new chromatophore centers had actually been formed. It was unquestionable that new red and blue pigments had formed where there was not a visible trace of a chromatophore at the beginning. Thus it is reasonable to suppose that red and blue pigments may arise quite independently of the yellow. The original yellow pigment now had a reddish tint instead of its former lemon yellow.

The blue network which gradually faded out during the 24 hours upon the white background reappeared during the next three days upon a white background, but now, instead of being a continuous network the blue pigment appeared in clumps around the red masses of pigment. This blue remained for two weeks in spite of the white background.

In this experiment the red appeared before the blue and the latter seemed always associated with the former pigment rather than with the yellow.

Formation and Destruction of White Pigment

A shrimp that was kept upon a white background for nearly two months had such a quantity of white pigment in its chromatophores that when this was dispersed it covered a considerable portion of the body. Due to the transparency of *Palæmonetes* white shrimps were ordinarily quite well concealed even upon a black background. However, when the white pigment had increased in quantity to the extent that it had in this instance, the shrimp stood out in sharp contrast when placed in a black dish. The stomach, due to the mass of white chromatophores upon it and its muscular contractions, fairly sparkled. Even after this shrimp had been upon a black background long enough for the white pigment to concentrate, the shrimp was still studded with clearly visible white spots. This was not altogether due to the quantity of white pigment present, but also to the absence of the red and blue pigments which would ordinarily assist in concealing it in such circumstances. It is such an example as this that makes me disagree with those workers in the past who have asserted that the white chromatophores play no part in color adaptation.

I have made no record of changes within an individual shrimp, but the general tendency of more and larger white chromatophores when a shrimp was long upon a white background, and fewer and smaller white

chromatophores in shrimps long upon a black background was striking enough to warrant this note of the fact.

Destruction of Blue Pigment

Although the origin of diffused blue pigment of *Palæmonetes* seemed to be the red pigment and the manner of its arrival in the tissues around the chromatophores and throughout the body seemed to be one of simple spreading, yet the disappearance of the pigment from the tissues appeared to be a question of destruction *in situ*. There has been no evidence that once the blue pigment was outside of the red pigment, there was any controlled migration back into it. An experiment was carried out to determine the effects of a white background and darkness upon the rate of destruction of the blue pigment.

Twenty-four deep blue shrimps were taken from a blue background and divided into two lots of twelve shrimps each. One of these lots was placed upon white background in the full light of a north window. The other lot was placed in total darkness. At the end of six hours the shrimps kept in the light had lost their blue coloration completely. In general appearance they were a yellowish white. The shrimps in darkness were promptly examined in a white light and found to be just as blue as at the moment when they were placed in the dark, but during the following six days there was a gradual disappearance of the blue network and the blue tint of all the tissues of the shrimps. Although blue was destroyed both in darkness and upon a white background in the light, the white background called forth a much more rapid destruction than did darkness.

A Quantitative Study of the Chemical Transformations in the Pigments

Hitherto no attempt has been made, as far as I am aware, to work out quantitatively the rates of pigment formation and destruction in crustaceans. A method is here set forth whereby quantitative determinations of the relative amounts of pigment in lots of shrimp subjected to various conditions can be made. The method is subject to some error due to the instability of the pigment itself, but since the results are relative and the technic uniform, it yields on the whole quite serviceable data. It gives one value which is the sum of the red, yellow, and blue pigments. The first step in the process is to convert the blue pigment to red by immersing the shrimps for a minute in boiling water. The work of Newbigin (1897) indicates that the red pigment that results from the heating of the blue is probably the same as the red that is present in the living shrimp.

The following is the technic that has been used in these experiments. Shrimps to be tested were dipped into boiling water for one to two minutes and then removed, cut into small pieces, and placed in a vacuum desiccator in darkness. At the end of 48 hours drying was completed and the dried shrimps were carefully weighed and then extracted with chloroform in the proportions of 30 cc. of chloroform to 1 gram dry weight of shrimps. The extraction was allowed to proceed for 24 hours, during which time the mixture was kept in a tightly stoppered test-tube in a refrigerator. After this interval of time the material was so well extracted that the remainder could be neglected. What would have been gained by a longer time of extraction would have been lost by the bleaching of the pigment. In the presence of light and air the bleaching occurred quite rapidly. Another source of error lay in the fact that the solvent was very volatile and thus the solution tended to concentrate in the course of the manipulations. Because of the volatility and photochemical activity of the material, the precautions taken during the drying and extracting were important ones.

At the end of 24 hours of extraction the solution was rapidly filtered and then the concentration of the pigment compared with that of a standard solution by the use of a colorimeter. Beer's law states that the amount of light transmitted by a solution varies inversely with the concentration of the absorbing substance. Hence a value for the concentration of the pigment in terms of the standard could be readily calculated. The standard solution was made by adding 1 cc. of methyl orange (.02 per cent in aqueous solution) to 16 cc. of an acetic acid sodium acetate buffer solution having a pH of 4.

One difficulty experienced in this method of obtaining values for the concentration was that different lots of shrimps contained different proportions of red and yellow pigments. This caused the solutions of pigment to differ from one another in tint. Considering that the colorimeter required the solutions compared to be the same color, some inaccuracy was introduced on this score. In order to determine the extent of this error several of the experiments were made and both the colorimeter and a spectrophotometer were used to measure the relative absorption of light by the solutions. The latter instrument was not affected by the color of the solution for here the light of only a definite wave-length was measured. The wave-length selected was 480 $m\mu$, the peak of the absorption spectrum of the pigment of these crustaceans. The results obtained by the two instruments checked each other very well. For most of the experiments the colorimeter was used alone because of the rapidity of operation.

When the effect of a given set of conditions upon the amount of

pigment in the shrimp was to be determined, a large number of shrimps were placed in the desired situation. At the beginning of the experiment and at three- or four-day intervals thereafter about twenty shrimps were taken from the lot as a sample to test for the concentration of the pigments. The value obtained was taken as a criterion of the concentration of pigment in the whole lot of shrimps at that particular moment. As this method rested to a great extent upon a statistical

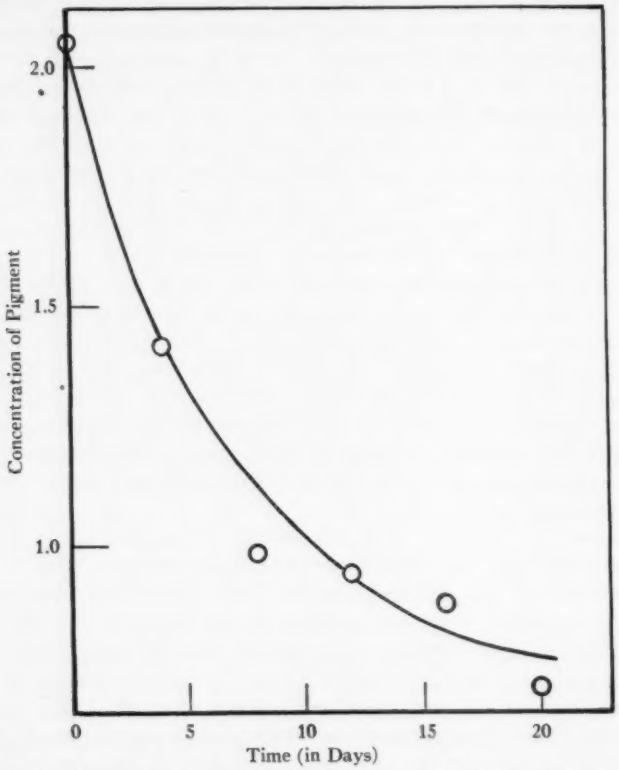


FIG. 3. Decrease in concentration of pigment in shrimps which were kept upon a white background in light. Concentration is stated in terms of a methyl orange standard solution.

basis, much of the irregularity of the results was attributed to the fact that so few shrimps were taken in each sample. There is little doubt that had the samples consisted of as many as fifty shrimps, the data exhibiting the effects of the environment upon the pigment content of the shrimps would have more closely fit a smooth curve when plotted.

Pigment Destruction upon a White Background

Approximately 250 shrimps were placed in a large white enamelled tub through which sea water was flowing. The tub was located in the center of a small room lighted by large north windows. During the course of the experiment the animals were fed upon finely chopped *Modiolus* and *Mytilus*. This experiment was repeated five times during the summer of 1932 with strikingly similar results. Figure 3 is typical of these experiments.

An experiment carried on in a similar manner except that the shrimps were starved over the period of twenty-four days yielded results showing the same rate of destruction of the pigments.

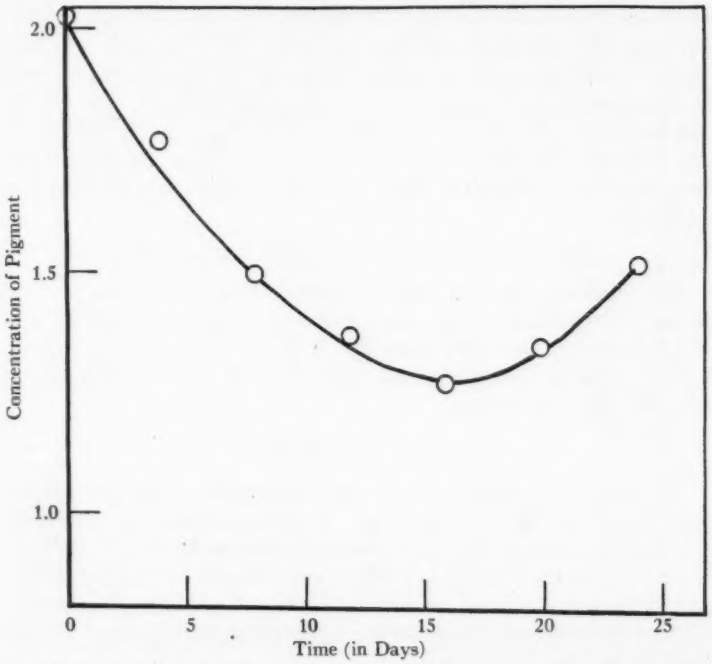


FIG. 4. Change in concentration of pigment in shrimps which were kept in darkness. Concentration is stated in terms of a methyl orange standard solution.

Effect of Darkness upon Pigment Quantity

In a second type of experiment shrimps were placed in large containers and allowed to remain in total darkness except for the brief moments when samples were being taken and the animals were being

fed. At these times a weak red light was turned on. This experiment was repeated six times during the summer. Sometimes the shrimps were fed and at other times starved during the course of the experiment. Again there was no difference between the results obtained when the shrimps were fed and when they were starved. This can be explained by the cannibalistic nature of the shrimps. Figure 4 is typical of the results.

The change from pigment decrease to pigment increase at the end of about fourteen days was constant in all the experiments and was quite curious. It does not agree with the observations of Brooks and Herrick (1891). These men found that *Palæmonetes vulgaris* was bleached nearly white after eighteen days in darkness. Their animals were sealed up during the entire period whereas mine were subjected to the occasional stimulation of the red light. This question will bear further investigation as it perhaps holds an explanation as to why deep sea crustaceans are generally heavily pigmented with red. In the deep sea it is known that the conditions are those of nearly total darkness. On the other hand, crustaceans which inhabit underground caves where there is an absence of light are usually conspicuous by their lack of pigment.

Pigment Formation upon a Black Background

In a third experiment a batch of partially bleached shrimps was put into large crystallizing dishes the outsides of which were painted with flat black paint. The dishes were left in the daylight of the laboratory. Figure 5 gives an example of the type of result that was obtained in four different experiments.

Control Experiment

As a control experiment for the preceding ones, a large glass aquarium was filled with sea water and in the aquarium was placed an abundance of the alga, *Fucus,* from which these shrimps were taken in nature. As nearly as could be judged, this situation very closely simulated the normal habitat of the shrimps both in color of the environment and in light intensity. The results taken from shrimps kept in this aquarium are seen in Fig. 6.

Discussion of Results

From the graphs of the experiments just described the following facts are evident. Upon a white background in the light there is a very rapid destruction of pigment within the shrimps. This destruction is continued over the twenty-day period of the experiment. In

another trial of the same nature the pigment was continuing to disappear even after thirty days. In this recorded case the pigment concentration decreased 65 per cent in the twenty days.

In darkness the rate of bleaching of the animal was considerably less than that seen upon a white background. This destruction of pig-

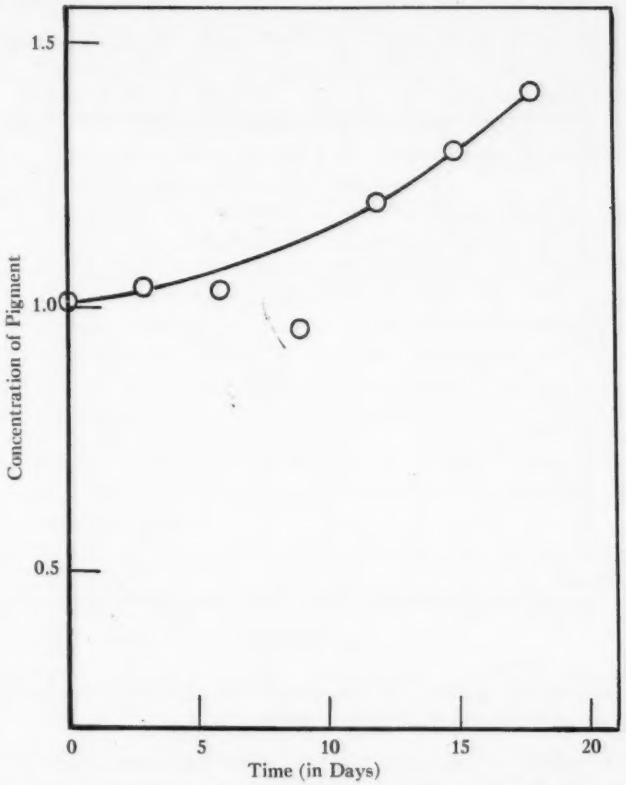


FIG. 5. Increase in concentration of pigment in shrimps which were kept upon a black background in light. Concentration is stated in terms of a methyl orange standard solution.

ment continued at approximately the same rate for sixteen days and then there was a reversal to pigment formation. In various experiments the time of this reversal varied from eleven to sixteen days after the animals were placed in darkness.

In the case of the partially bleached shrimps that were placed upon

a black background there was at first only a slow formation of pigment and then an increase in rate. The rate continued to increase over the eighteen-day period of the experiment. During this time there was a 40 per cent increase in the amount of pigment in the shrimps.

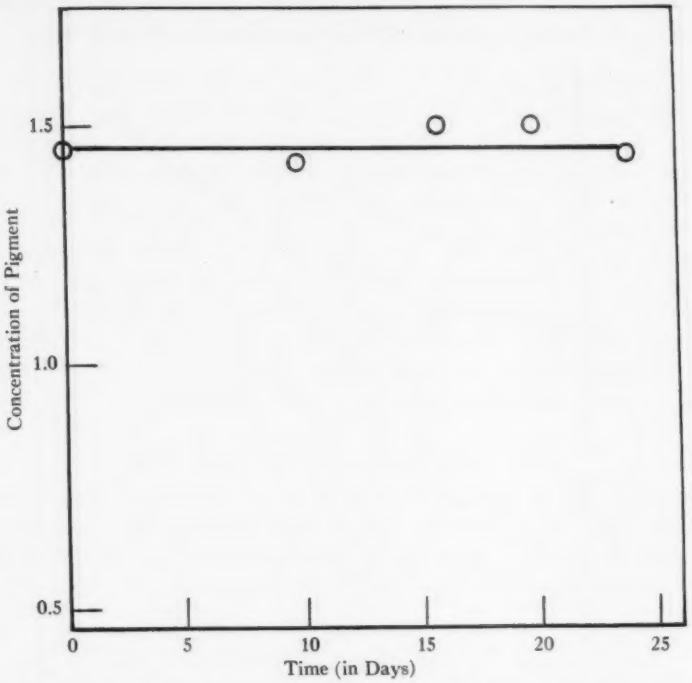


FIG. 6. Condition of concentration of pigment in shrimps which were kept in an environment which closely simulated the normal habitat. Concentration is in terms of a methyl orange standard solution.

The validity of this experimental technic was again checked in observing the behavior of the concentration of pigment of shrimps that were kept in apparently normal conditions. Here, over a period of twenty-four days, an interval of time as great as that of the longest experiment, there was neither an increase nor a decrease in the concentration of pigment.

It was shown by separating the extracted pigment of the animals into its two components by shaking a petroleum ether solution of the pigment with a strong solution of potassium hydroxide in 90 per cent methyl alcohol, that the shapes of these curves illustrating the rates of

formation and destruction of pigment were almost wholly effected by the increase and decrease in the amounts of the red and blue. The rate of change of the quantity of the yellow pigment was relatively insignificant.

Controlling Mechanism of Pigment Formation and Destruction

Keeble and Gamble (1904), who were at the time working upon the prawn, *Hippolyte varians,* put forth an hypothesis to account for pigment formation and destruction. Since that time this hypothesis has been restated by other investigators such as Babák (1913), and Odiorne (1933). In substance it states that a dispersed pigment is increasing in amount while a concentrated one is decreasing.

The observations in this report confirm the hypothesis originated by Keeble and Gamble. For example, on a white background there is a strong concentration of the red and yellow pigments, an absence of diffusing blue, and a strong dispersion of the reflecting white pigment. Correspondingly there is an increase in the amount of white pigment and a decrease in the amounts of the other three pigments. A black background, just as the hypothesis calls for, brings about an increase in the quantities of the red, yellow, and blue pigments and at the same time a decrease in the amount of white pigment within the chromatophores. Darkness causes a destruction of red pigment at about half the rate seen in an animal upon a white background in the light and here the red pigment is slightly dispersed.

On the basis of these facts it is possible that pigment formation and destruction in adaptation to colored backgrounds may be controlled by the same humoral agents that are responsible for the control of migration of the pigments within the chromatophores.

I take this opportunity to acknowledge my indebtedness to Dr. G. H. Parker and Dr. A. C. Redfield for their invaluable criticisms and suggestions.

Summary

The red pigment of *Palæmonetes vulgaris* appears to be astacin, while the yellow seems to be identical with plant carotin.

A method is set forth to measure the rate of formation and destruction of pigment within the shrimps. It is observed that the red and blue pigments are most rapidly formed and destroyed, while the yellow and white pigments are much more slowly changed.

Upon a white background in the light the red and blue pigments are destroyed very rapidly. Yellow disappears slowly.

Upon a black background in the light the red and blue pigments are formed.

Shrimps kept in darkness at first lose through destruction their red and blue pigment but after about two weeks there occurs a reversal of this process (pigment formation).

LITERATURE CITED

BABÁK, E., 1913. Über den Einfluss des Lichtes auf die Vermehrung der Hautchromatophoren. *Arch. ges. Physiol.*, **149**: 462.

BROOKS, W. K., AND F. H. HERRICK, 1891. The Embryology and Metamorphosis of the Macroura. *Mem. Nat. Acad. Sci.*, **5**: 323.

FABRE, R., AND E. LEDERER, 1934. Contribution à l'étude des lipochromes des animaux. *Bull. Soc. Chim. Biol.*, **16**: 105.

FRÖHLICH, A., 1910. Farbwechselreactionen bei Palaemon. *Arch. Entw.-mech.*, **29**: 432.

KARRER, P., AND L. LOEWE, 1934. Constitution of Astacin. *Nature*, **133**: 986.

KEEBLE, F. W., AND F. W. GAMBLE, 1904. The Colour-Physiology of Higher Crustacea. *Phil. Trans. Roy. Soc. London, B*, **196**: 295.

KUHN, R., AND E. LEDERER, 1933. Über die Farbstoffe des Hummers (Astacus gammarus L.) und ihre Stammsubstanz, das Astacin. *Ber. deut. Chem. Gesell.*, **66**: 488.

LÖNNBERG, E., AND H. HELLSTRÖM, 1932. Zur Kenntnis der Carotinoide bei marinen Evertebraten. *Arkiv. för Zoologi*, **23**: 1.

MCNICHOLAS, H. J., 1931. The Visible and Ultraviolet Absorption Spectra of Carotin and Xanthophyll and the Changes Accompanying Oxidation. *Bureau Stand. Jour. Res.*, **7**: 171.

NEWBIGIN, M. I., 1897. The Pigments of the Decapod Crustacea. *Jour. Physiol.*, **21**: 237.

ODIORNE, J. M., 1933. Degeneration of Melanophores in Fundulus. *Proc. Nat. Acad. Sci. Wash.*, **19**: 329.

REDFIELD, A. C., 1930. The Absorption Spectrum of Some Bloods and Solutions Containing Hemocyanin. *Biol. Bull.*, **58**: 150.

HISTOLYSIS AND REGENERATION OF ANURAN TAIL SKIN [1]

F. W. DUNIHUE

DEPARTMENT OF BIOLOGY, UNIVERSITY COLLEGE, NEW YORK UNIVERSITY

INTRODUCTION

Atrophy of the larval tail is perhaps the most readily observed phenomenon occurring during anuran metamorphosis. Descriptions of the progressive disappearance of this organ are usually limited, however, to the brief statement that the dorsal and ventral finny portions of the tail are the first to be resorbed, followed closely by the median region. Such a conception is entirely in accord with the atrophic changes in the tail of the salamander, for during the metamorphosis of this amphibian the dorsal and ventral tail-fins disappear while the median portion persists. The process, however, aside from its antero-posterior progression in the anurans, has received little if any critical study, with the result that the current view is based upon more or less cursory macroscopic observations.

The factors inducing the resorption of the larval anuran's tail, on the other hand, have received much more attention. Barfurth (1887) believed the atrophic changes to be due to a cutting off of the vascular supply to the tail, brought about by pressure of the growing urostyle upon the dorsal aorta. This idea was challenged by Bataillon (1891) and Mercier (1906). The former attributed the histolytic changes to a rearrangement of the capillary system in the tail, while the latter held that occlusion of the aorta by the urostyle was only partial. Morse (1918) and Bradley (1922), however, accepted Barfurth's suggestion as quite logical, and stated that such an occlusion, whether complete or partial, would result in the accumulation of CO_2 and various acid metabolites, causing an activation of the autolytic enzymes. Helff (1930) has recently demonstrated that removal of the urostyle anlage in larval forms does not alter in any particular the typical atrophy of the tail during metamorphosis. He pointed out that, such being the case, the urostyle could no longer be considered the fundamental factor inducing tail atrophy, and that the initiatory agent is probably of a more general nature. The investigations of Helff (1926), Lindeman (1929), Helff

[1] This paper, together with Part II of this series of studies, was submitted to the Graduate School of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, April 1, 1934.

and Clausen (1929), and Clausen (1930, 1932) on the histolysis and regeneration of anuran skin and muscle transplantations all indicate that the initiation and maintenance of tail histolysis may be due to specific substances in the blood stream, or to a general lowering of the blood pH. Evidence in support of this latter factor has been advanced by the work of Helff (1932*a*) in which he found a definite drop in blood pH during larval involution.

In the course of his studies on skin transplantation, Lindeman (1929) observed that tail-skin grafts, placed near the antero-posterior center of the larval tail, underwent an apparent migration anteriad during metamorphosis. Schubert (1926) noted the same phenomenon in the case of hind-limb buds transplanted to the tail. Such observations suggested that the anterior levels of the tail are more susceptible to the histolytic influence than the more posterior levels or, in other words, that the proximal regions of the tail are resorbed before the more distal. This possibility was investigated by Clausen (1930) and found to be the case. An antero-posterior gradient of susceptibility to histolysis was shown to hold for both tail-skin and muscle. In addition to supporting the contention that the histolytic factor is present in the blood, the findings of Clausen indicate that tail tissues possess a threshold value for the stimulus inducing atrophy which is specific for any given antero-posterior level.

Spallanzani, as early as 1769, reported that the amount of tissue regenerated after amputation of the tadpole tail at different levels varies with the amount removed. Many later workers (Barfurth (1894), Morgan (1906), Ellis (1909), Durbin (1909), and Zeleny (1916)) have verified this observation, and, in more detailed analyses, found that the more anterior, or cephalic the level of injury, within certain limits, the greater the rate of regeneration. These writers seem to be in agreement that the differences in regenerative rate of tail tissue *in situ,* at different levels, are not due to specific qualities resident in the cells. Morgan (1906) proposed that a change in the pressure relationships of the part concerned is the stimulus for regeneration; division proceeding until intracellular and extracellular tensions come to equilibrium. Ellis (1908, 1909) accepted the above explanation as being consistent with experimental data, while Zeleny (1916) suggested that regeneration "must be under some central control, probably connected with general functional activity." More recently, Clausen (1930), in studying the regeneration of transplanted partially histolyzed skin from four levels of the antero-posterior axis of the tadpole tail, has approached this problem from another angle. His results established an antero-posterior gradient in regenerative rate for integument following partial histolysis,

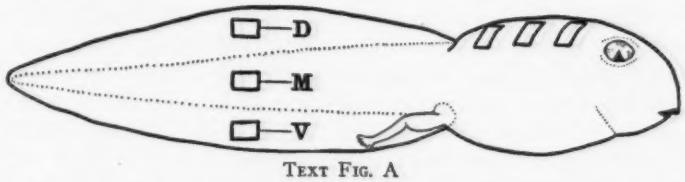
and indicated that this gradient in regenerative rate is due to factors inherent in the skin.

The purpose of the present investigation was threefold; first, to see whether or not a gradient of susceptibility to histolysis is present in integument from the dorso-ventral axis of the tadpole tail; second, to ascertain whether or not partially histolyzed skin, from different regions of the same axis, possesses a specific regenerative rate typical of integument for the level in question; and third, to determine the bearing of the results obtained on the mechanism of tail atrophy.

The writer is indebted to Professor O. M. Helff of this laboratory for suggesting the problem and for his direction during the course of the investigation.

Material and Methods

The stock used for all operations was large *Rana catesbiana* tadpoles measuring 105–115 mm. in length, with hind legs 4–8 mm. in length. The tadpoles were obtained from ponds in southern New Jersey and Long Island during 1932–33. They were kept in large aerated aquaria for a week or more in order to eliminate the less hardy specimens. All individuals selected for use were, insofar as could be determined by external indications, normal larvæ which remained unchanged under laboratory conditions.



Text Fig. A

The technique used in making the autotransplantations was as follows: After anesthetization in a 0.05 per cent aqueous solution of chloretone, the animal was placed on the stage of a dissecting microscope and rectangular pieces of integument, 3 by 5 mm. in size, accurately cut from the dorsal, median, and ventral regions of the tail at the level of its greatest dorso-ventral width (*D*, *M*, and *V*; text figure *A*). They were then carefully transplanted to areas on the back from which the skin had previously been removed (text figure *A*). The linear sequence of the grafts on the back was varied to obviate any possible effects due to the site of transplantation. Each animal was placed in an individual bowl immediately after the operation and allowed to remain 15 to 30 minutes without water. This tended to facilitate wound healing and prevent washing off of the transplant. At the end of this period the

jar was filled with water and the specimen maintained under constant laboratory conditions for subsequent observations.

Following a healing period of seven days the grafts were measured under the dissecting microscope and the dimensions recorded. The areas computed from these measurements were considered as original areas of the grafts from which percentage reductions were later calculated. This procedure was necessary because the dimensions of the transplants changed slightly during the time allotted for healing. Those individuals in which one or more grafts failed to heal properly were discarded. The number of such cases, however, was quite small. Larval metamorphosis was induced at this time by feeding desiccated thyroid. Daily observations were made and the water changed as often as needed to keep it fresh. The grafts were measured, under the dissecting microscope, once each week during the early stages of involution and every third day in the later stages. At the arbitrary periods of 20, 50, and 80 per cent reduction in surface area, several of the transplants of each tail region were fixed in Bouin's fluid for sectioning. They were subsequently stained with Ehrlich's hematoxylin and counter-stained with eosin.

Results

Normal Tail Integument

The normal tail integument of *Rana catesbiana* does not differ materially from that of other larval anurans, and for this reason a detailed account of its structure is unnecessary. As an aid to the interpretation of the degenerative and regenerative processes, however, the main structural features will be reviewed briefly. Macroscopically, the tail-skin is translucent and of a very fine texture in contrast with the opaque, coarse nature of back-skin. With respect to pigmentation, the tail-skin contains more xantholeucophores and fewer melanophores than back-skin (Fig. 1).

Histologically, the integument can be resolved into three strata; the epidermis, the dermis, and the subcutis (*E, SC,* and *CT*; Fig. 5). The epidermis is composed of two or three layers of cells and an outer cuticular border. The cells of the outermost layer are low columnar to cuboidal in shape. The basal cells range in form from medium to tall columnar, and have within the cytoplasm fibrous, brush-like structures which are attached to the basement membrane (see *FS*; Fig. 5). Occasional mitotic figures as well as a few melanophores are present in this layer. One of the most striking histological differences between tail-skin and back-skin is found in the structure of the dermis. In tail-skin this consists of a single layer, the stratum compactum, but in back-skin

there is in addition the stratum spongiosum, a region of loose connective tissue lying between the stratum compactum and epidermis. The stratum compactum of tail-skin is relatively thin and structurally quite homogeneous, whereas that of back-skin is formed by an upper layer of loosely arranged fibers and a denser more compact lower layer. Immediately beneath the dermis is the subcutis, consisting of loose connective tissue fibers and cells, lymphocytes, capillaries, and chromatophores.

The Degenerative Process

The sequence of degenerative changes in the integument is quite regular. Consequently, regardless of the source of the transplants, their histolytic changes are identical when the degree of resorption, as measured by the reduction in surface area, is the same. Therefore, the following description of the histolytic process is applicable to transplants from any one of the three regional sources used. The macroscopic changes, aside from a reduction in surface area, are confined to the pigmentation of the graft. When reduced 20 per cent the transplants are only slightly darker than normal skin (Fig. 2). They are considerably darker at 50 per cent reduction, with only a few or no xantholeucophores present (Fig. 3). When histolyzed 80 per cent they are of a uniform shade and very dark. In this later stage definite pigmentation spots may entirely disappear (Fig. 4). With continued degeneration the graft becomes smaller and darker in color, until finally it is completely resorbed and nothing remains but a dark scar indicating the line of fusion of the surrounding back-skin. To summarize: the depth of coloration is directly proportional to the degree of histolysis.

A detailed study of the progressive steps in the histological disintegration has not been attempted, but rather three arbitrary stages have been selected for microscopic examination, namely: when the transplants were 20, 50, and 80 per cent reduced in surface area. In the first stage the major changes are centered in the epidermis. This layer is thicker due to an increase of one or two cell layers, coupled with hypertrophy of the basal cells. The latter factor is in itself sufficient to account for the increased thickness in some cases (Fig. 6). The height of the outermost stratum of cells, as well as the cuticular border, is reduced about one-half as compared with the normal condition. Other signs of histolysis are seen in the fading of the fibrous, brush-like structures of the basal cells, and the invasion of lymphocytes into the epidermis. The dermis (stratum compactum) undergoes a slight reduction in thickness, exhibiting a few lymphocytes imbedded among the fibers. There is an increase in the cellular elements of the subcutaneous tissue; mainly lymphocytes but also a few eosinophilic leucocytes.

With the surface area reduced 50 per cent the histological structures have undergone extensive changes. The epidermis, while still consisting of two or three cell layers, is very greatly reduced (compare Figs. 6 and 7). The cuticular border of the now very much flattened cuticle cells has entirely disappeared. The fibrous, brush-like structures of the basal cells are no longer visible. In short, the epidermis has come to resemble, except for an occasional lymphocyte, the surrounding back-skin. Perhaps the most remarkable change is the almost complete resorption of the stratum compactum, which is represented by a very thin, loose, fibrous layer (*SC*, Fig. 7). The underlying layer of connective tissue contains a considerable number of blood cells, especially lymphocytes, but fewer than were present in the preceding stage.

When the transplant is reduced 80 per cent in surface area profound histological changes have occurred. It is very difficult to determine the structure of the epidermis in this stage, owing to the large number of melanophores aggregated in this layer (*ML*, Fig. 8). Furthermore, cellular boundaries are indistinct, and the nuclei vary widely in shape, size, and distribution. The cuticular stratum of cells is much thicker than in the foregoing stages, due to the inclusion of underlying cells from the intermediate layer. It contains one or two layers of flattened, ellipsoidal nuclei. Briefly, the epidermis presents a picture of great disorganization (*E*, Fig. 8). The original stratum compactum has disappeared, as well as much of the subcutis. The partial loss of the subcutaneous layer, it seems, results from the formation, underneath, of a thick stratum compactum derived from the stratum compactum of back-

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PLATE I

FIGS. 1–4. Sketches illustrating changes in the macroscopic appearance of autotransplanted tail-skin during metamorphosis. × 7.

FIG. 1. Autoplastic tail-skin graft seven days after transplantation to the back of a non-metamorphosing larva. No apparent histolysis.

FIG. 2. Macroscopic appearance of autotransplanted tail-skin, on the back of a metamorphosing larva, when reduced 20 per cent in surface area.

FIG. 3. Macroscopic appearance of autotransplanted tail-skin, on the back of a metamorphosing larva, when reduced 50 per cent in surface area. Note darkening of graft.

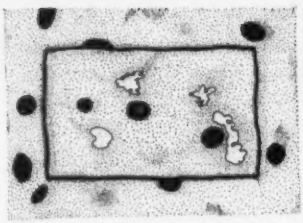
FIG. 4. Macroscopic appearance of autotransplanted tail-skin, on the back of a metamorphosing larva, when reduced 80 per cent in surface area. Uniformly very dark.

FIGS. 5-6. *CB*, cuticular border; *CL*, cuticle cell layer; *E*, epidermis; *FS*, fibrous brush-like structure; *SC*, stratum compactum; *CT*, subcutis; *ML*, melanophore; *FB*, fibroblast; *LM*, lymphocyte; *EL*, eosinophilic leucocyte; *ER*, erythrocyte.

FIG. 5. Section through normal tail-skin. × 510.

FIG. 6. Section through autoplastic tail-skin graft when reduced 20 per cent in surface area. The thickened epidermis, reduced cuticular border and cells are characteristic of this histolytic stage. An increase in number of lymphocytes and invasion by a few eosinophilic leucocytes are the principal changes in the subcutis. There is a slight reduction in thickness of the stratum compactum. × 510.

PLATE I



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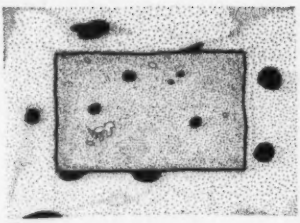

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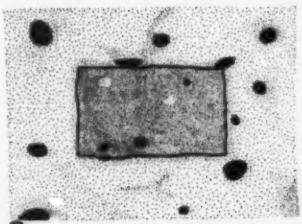

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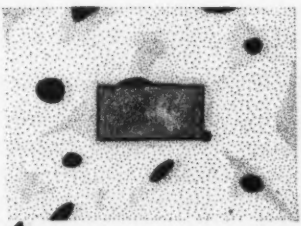

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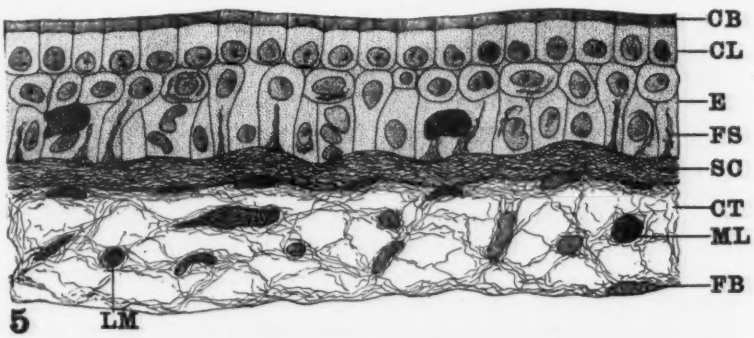

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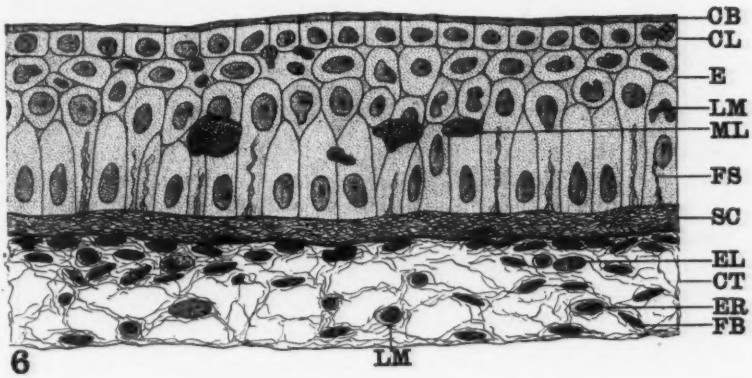

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skin. This newly formed stratum compactum is separated from the epidermis near the borders of the transplant by remnants of the subcutis, but in the central regions it is contiguous with the epidermis. It should be emphasized that this secondary stratum compactum does not possess the typical structure of that found in back integument. Although it is fully as thick as in the latter case, it is approximately of the same density at all levels, while back-skin compactum is composed of an upper loosely knit region and a lower more dense portion. Moreover, it is in intimate contact with the epidermis at some points, without the intervening stratum spongiosum so typical of back-skin.

Median-Ventral-Dorsal Sequence of Susceptibility to Histolysis

The basis for determining possible susceptibility gradients to histolysis in integument from the dorso-ventral axis of the tail was a comparison of the percentage reductions in the surface areas of the transplants. Supplemental histological examinations were also made. The case history of the grafts on one individual, from the time of transplantation until death of the animal, will illustrate the progression of histolysis on this basis (Table I). The letters *D, M,* and *V* of the table designate the regional source of the transplants, referring to dorsal, median, and ventral portions of the tail, respectively (see text fig. *A*). This table shows that histolysis is first evident in the median graft, next in the ventral, and finally in the dorsal. Furthermore, when this particular host animal died, *M* was completely histolyzed, *V* 80 per cent, and *D* only 56 per cent. It seems quite probable that the grafts would have disappeared, had the animal lived, in the order *M–V–D,* especially since that was the order of histolysis throughout the previous stages. The results do not warrant a definite conclusion on this matter, however, since in only one case did an animal live long enough for the complete histolysis of more than one graft. In this individual the ventral graft was the second one to disappear. Observations on the changing coloration of the transplants also corroborate the sequence of histolysis, *M–V–D*. The *M* graft was the first to become darkened, followed in order by *V* and *D*.

For a comparative study of histolysis in the various transplants the percentage reductions in surface areas were tabulated at four arbitrary periods; namely, when *M* was 20 per cent reduced, when *V* was reduced 20 per cent, when *D* was 20 per cent reduced, and when *M* was completely histolyzed or almost so. The results for each particular period were found to be quite uniform with only slight variations from the average. A summary of this extended tabulation is given in Table II and it will be noted that the histolytic sequence of the grafts at each of

TABLE I

Histolysis of tail-skin transplants. Case history DMV_{33}. Transplanted January 16, 1933.

Date of Measurements	Percentage Reduction in Surface Area		
	Regional Source of Grafts *		
	D	*M*	*V*
Feb. 9	0	16	0
Feb. 16	0	21	0
Feb. 25	0	40	8
Mar. 2	0	43	14
Mar. 7	6	47	20
Mar. 16	19	55	46
Mar. 22	23	55	48
Mar. 27	30	65	50
Apr. 7	42	80	66
Apr. 15	44	88	74
Apr. 17	56	100	80

* The letters *D*, *M*, and *V* refer to the dorso-ventral levels of the tail from which the transplants were obtained (See text fig. *A*).

these four stages, as indicated by the percentage reductions in surface area, is median-ventral-dorsal. Exceptions to this order of histolysis were noted in stage three (*D* reduced 20 per cent). At this time *V* was occasionally slightly more reduced than *M* and continued so for a short interval. However, in the earlier and later stages *M* was invariably histolyzed to a greater extent.

TABLE II

Comparative Histolysis of Tail-Skin Transplants

Regional source *	Average Percentage Surface Area Reduction			
	When M is reduced about 20% (183 cases)	When V is reduced about 20% (119 cases)	When D is reduced about 20% (50 cases)	When M is reduced 100% (10 cases)
D	0.7	4.36	20.8	68.0
M	21.9	46.9	49.4	100.0
V	1.67	20.9	48.3	84.0

* The letters *D*, *M*, and *V* refer to the dorso-ventral levels of the tail from which the transplants were obtained (See text fig. *A*).

Although the microscopic data are not as comprehensive as the macroscopic data, they are in close agreement with the results recorded in the tables. Consider, for example, the three grafts on one individual when *V* is reduced 20 per cent in surface area. The histological pic-

ture of *V* at this stage is shown in Fig. 6; *M* which is 50 per cent reduced is illustrated in Fig. 7; and *D*, having undergone only very slight reduction, shows little or no variation from normal integument (Fig. 5).

Regenerative Sequence of Partially Histolyzed Integument

In studying the rate or incidence of regeneration of partially histolyzed skin from the dorso-ventral axis of the tail, grafts reduced 20 and 50 per cent in surface area were secured according to the method described in the foregoing section of this paper. They were then transplanted homoioplastically to the backs of normal larvæ which would remain as such, under laboratory conditions, for at least six months. Approximately one hundred and eighty transplantations of this type were made. Beginning sixteen days after the second (homoioplastic) transplantation, four grafts of each of the three types (*D*, *M*. and *V*; 12 in all) were fixed at each successive three-day interval for histological examination. Although the degree of histological degeneration in grafts reduced 20 and 50 per cent in surface area had been determined in the previous experiments, additional cases were sectioned for controls at the time of homoioplastic transplantation.

The progression of regeneration, as with histolysis, is very regular, and, regardless of the source of the integument, the regenerative changes of any two grafts will be the same at any specific degree of regeneration. It will be necessary, then, to describe the process in integument from only one of the three source levels. Since regeneration is more extensive in skin histolyzed 50 per cent, in consequence of the greater histolytic disintegration, it has been selected as the type example (Fig. 7). It must be borne in mind that the chief aim of this investigation

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PLATE II

FIGS. 7–10. *CB*, cuticular border; *CL*, cuticle cell layer; *E*, epidermis; *FS*, fibrous brush-like structure; *SC*, stratum compactum; *CT*, subcutis; *ML*, melanophore; *FB*, fibroblast; *LM*, lymphocyte; *EL*, eosinophilic leucocyte; *ER*, erythrocyte.

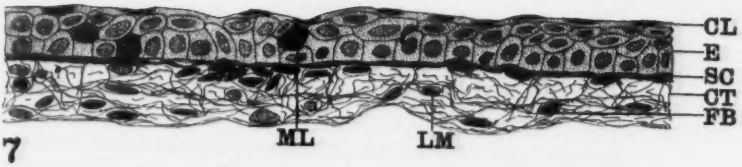
FIG. 7. Section through autoplastic tail-skin graft when reduced 50 per cent in surface area. The cuticular border and fibrous, brush-like structures have disappeared. The cuticular cells and stratum compactum are greatly reduced. × 423.

FIG. 8. Section through autoplastic tail-skin graft when reduced 80 per cent in surface area. The epidermal layer is considerably disorganized and contains many melanophores. The stratum compactum is obliterated. × 510.

FIG. 9. Section through homioplastic graft 28 days after transplantation. Regeneration is manifest in the reëstablishment of the cuticular border, cuticle cells, and fibrous brush-like structures. The stratum compactum is being reformed. × 510.

FIG. 10. Section through homioplastic graft 37 days after transplantation. The cuticular border, cuticle cells, and epidermis in general are almost normal. Complete regeneration of the subcutis and stratum compactum requires about six more days. × 510.

PLATE II



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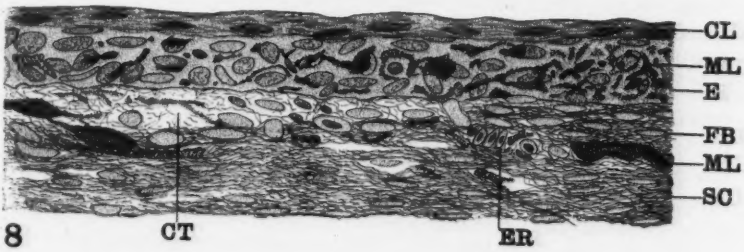

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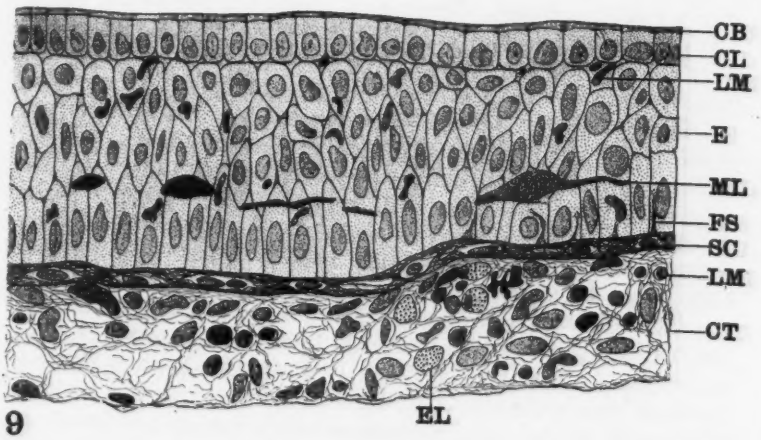

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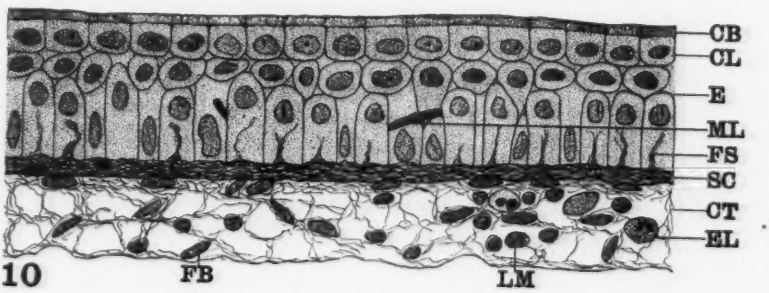

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was to determine the time necessary for complete reconstruction to occur, rather than to make a comprehensive study of the regenerative process.

The regenerative process begins a few days following homoiotransplantation. This is first indicated by an increased thickness of the epidermal layer. The exact mechanism of this increase has not been ascertained because the condition was well established prior to the first fixation period (16 days). However, since the area of the transplant is not appreciably reduced at this time, it seems unlikely that the epidermal thickening is due to a crowding or pressure effect of the surrounding back integument. After a regenerative period of 28 days the epidermal layer is several times thicker than it was at the time of transplantation, and has been invaded rather sparingly by lymphocytes (Fig. 9). A reëstablished cuticular border, although somewhat reduced, and the restored columnar form of the cuticular cells are characteristic of the epidermis at this stage. In the basal cells the fibrous, brush-like structures are being reformed. The stratum compactum has become prominent once more, containing lymphocytes and fibroblasts imbedded among its fibers. The subcutis contains several lymphocytes and a few eosinophilic leucocytes. Other blood elements are quite probably present but the staining technique used was inadequate for a critical determination of these cells. With continued regeneration the component parts of the epidermis gradually return to normal; first the cuticular border and cuticle cells, then the intermediate and basal layer of cells, and finally the fibrous, brush-like structures of the basal cells. Meanwhile, the fibers of the stratum compactum become more firmly bound together, with very few cells among them. The cellular elements of the subcutaneous tissue decrease in number so that, except for a few more lymphocytes and an occasional eosinophil, it closely resembles the normal subcutis. Figure 10 shows such an intermediate stage in which the epidermal layer is almost normal, with the stratum compactum and subcutis rapidly nearing complete reconstitution. Further growth of the stratum compactum and diminution in the number of lymphocytes in the subcutis, accompanied by the disappearance of the leucocytes, restores the original normal structure of the integument (Fig. 5).

The appearance of the homoiotransplants undergoes very little alteration during the greater part of the regenerative period. Usually within six to nine days before complete regeneration, however, the normal coloration returns, although the grafts may sometimes become lighter in color at an earlier stage. This rather sudden change in depth of coloration near the close of the regenerative process is probably correlated with the withdrawal of cellular elements (lymphocytes and leucocytes)

from the subcutis and a decrease in the number of cell layers in the epidermis. A re-arrangement of the melanophores is also a contributing factor. Another point of considerable interest is that at no time after a transplant had properly healed was it replaced by the surrounding back-skin.

TABLE III

Regeneration of Tail-Skin Transplants

Regional Source *	Initial Reduction in Surface Area	Days Taken for Return of Normal Histolytic Picture				Average Time
		Case 1	Case 2	Case 3	Case 4	
	per cent					
D	20	35	37	37	40	37
	50	49	52	52	49	50
M	20	28	31	31	31	30
	50	40	43	43	40	41.5
V	20	31	34	34	37	34
	50	43	46	46	49	46

* The letters *D*, *M*, and *V* refer to the dorso-ventral levels of the tail from which the transplants were obtained (See text fig. *A*).

Histological examinations revealed that complete reconstruction, in both 20 and 50 per cent histolyzed grafts, occurs in the median transplant before it does in either the ventral or dorsal, and in the ventral before the dorsal. That is, the three different regional grafts do not achieve complete regeneration at the same time but in the order median-ventral-dorsal. These results are set forth in Table III. The data in this table give only the results for complete reconstitution, but it should be borne in mind that this same sequence holds for any intermediate stage. In other words, *M* is the first graft to attain any given stage of regeneration, succeeded in order by *V* and *D*. Thus, partially histolyzed integument from the dorso-ventral axis of the tadpole tail possesses a sequence either in the time of regenerative incipience or the rate of regeneration which is of the same order as the histolytic sequence, namely, median-ventral-dorsal.

DISCUSSION

The results are clear in that integumentary grafts from different dorso-ventral levels of the tail do not histolyze simultaneously but in

the sequence median-ventral-dorsal. The cause of this histolytic sequence is not entirely clear; it may be due to a time or rate differential, or a combination of these two factors. In the initial stages, however, it is unquestionably the former, since signs of histolysis are first evident in the median graft, next the ventral, and finally the dorsal. This order of histolytic incipience was true for all but nine of the 183 cases studied. In these exceptions the only change was a reversal in the order of appearance of histolysis in the ventral and dorsal grafts. It may be that in the later stages of histolysis a rate differential becomes a factor in the maintenance of this sequence. Since the histolytic rate of each graft is characterized by alternating periods of acceleration and deceleration, it is difficult to ascertain possible rate differences. In fact, rate changes in any one graft appear to be roughly equal to those occurring in other regional grafts. For this reason the writer is inclined to believe that the described sequence is largely the result of a difference in the time of histolytic incipience and not due to differential rates.

Since grafts from different dorso-ventral regions of the tail complete histological regeneration in the order median-ventral-dorsal, it is obvious that tail-skin possesses a regenerative as well as an histolytic sequence. The relative time required for each regional graft to restore its normal histological structure was the basis used in determining this sequence. An exceptionally large number of homoiotransplantations were necessary in making this determination because of the comparatively long regenerative period of partially histolyzed integument. In fact, sixty 50 per cent histolyzed grafts, each involving two transplantation procedures, were required to determine the time necessary for complete histological reconstitution of the median transplant alone. Consequently, the grafts were not fixed, for sectioning and subsequent examination, in the earlier regenerative stages. The first fixation period, for the most part, was sixteen days after the second (homoioplastic) transplantation, at which time regeneration had begun in all three regional grafts. For this reason the question of whether or not the sequence is due to a difference in the time of regenerative incipience, rate, or a combination of these factors cannot be answered without a more detailed study. The histological sections of such early stages as were made, however, indicate that the time of regenerative incipience is not the same for each regional graft. In view of this it may not be entirely out of place to suggest that further work may show the regenerative differential to be dependent largely, if not entirely, upon differences in the time of regenerative incipience.

In the regenerative series of experiments no incompatibility between homoiotransplants and host could be detected after the grafts had healed.

There were a few cases in which the grafts failed to "take," but in most cases this was probably due to errors in transplantation technique. The pigmentation and general appearance of the homoioplastic transplants were at all times characteristically those of tail-skin. Herrick (1932), on the other hand, reported that homoiotransplants of side and belly-skin acquire typical back-skin pigmentation, and hence concluded that homoioplastic grafts are replaced by host tissues. He failed to find any evidence, however, for an overgrowth or undergrowth of the transplant by host epidermis as suggested by Cole (1922). In the present work there were no indications of an invasion of the graft by host epidermis. Furthermore, the stratum compactum seems to be restored by new formation in and under the old graft layer, a condition previously noted by Helff (1926). Moreover, the regenerated stratum compactum is of the typical tail-skin type even though surrounded by the distinctly different back-skin compactum. It is concluded, therefore, that in the present experiments an actual regeneration of the transplanted integument occurred, which did not involve replacement by host tissues.

The fibrous, brush-like structures of the epidermal cells agree in their morphology, and in their reactions to degenerative and regenerative stimuli, with the "coarse mitochondrial threads" described by Speidel (1926). Since the technique used did not permit of a critical cytological study of these elements, there is no evidence either for or against Saguchi's (1913) theory that they are fused mitochondria. Their principal interest in the present investigation lies in their characteristic behavior during the regeneration and degeneration of the integument.

Granting that histolytic and regenerative sequences do exist in tail integument, it is interesting to inquire as to their physiological basis. Since tail-skin after transplantation to the back histolyzes during metamorphosis, the stimulus inducing atrophy must be present in the general circulation, a condition long advocated by Helff et al. The histolytic sequence of grafts from different dorso-ventral levels in the order median-ventral-dorsal, then, can only be due to inherent differences in the skin, for each graft is subjected to the same atrophic stimuli. Likewise, the restitutional phenomena occurring in partially histolyzed tail-skin grafts must also be dependent upon inherent qualities of the integument. Such grafts, when removed from metamorphosing larvæ and transplanted to the backs of non-metamorphosing tadpoles, complete regeneration in the sequence median-ventral-dorsal. This sequence of regeneration cannot be due to differences in environmental conditions for all grafts were acted upon by the same external forces, the result of a common transplantation site. The logical interpretation is that tail integument possesses regenerative potentialities which differ for each

dorso-ventral level. Any statement as to the basis for these inherent potentialities is certain to be somewhat speculative, but Helff's (1932*b*) recent demonstration that the anterior levels of the tadpole tail are electro-negative to the more posterior regions suggests a possible explanation. Although it is generally agreed that potential differences arise as a result of differential metabolic rates, there is considerable disagreement as to whether the region of higher rate is electro-negative or electro-positive to the region of lower rate. However this may be, the important point is that there is a difference in potential between the cephalic and caudal regions of the tadpole tail which, according to the above interpretation, in turn indicates the existence of a metabolic gradient. On this basis the writer suggests, tentatively, that the inherent histolytic and regenerative potentialities of tail integument, as evidenced by the present work, are determined or conditioned by differences in metabolic rate characteristic of the three dorso-ventral tail regions studied.

Summary

1. Uniform-sized tail-skin grafts were secured from extreme dorsal, ventral, and one intermediate region of the tail and transplanted, autoplastically, to the backs of non-metamorphosing *Rana catesbiana* tadpoles. Artificial metamorphosis was then induced and the histolysis or reduction of the grafts studied. The median-source graft was invariably the first to undergo reduction followed in order by the ventral and dorsal, regardless of their antero-posterior transplantation sequence on the back.

2. Similar grafts of tail-skin were transplanted, autoplastically, to the backs of non-metamorphosing larvæ, and artificial metamorphosis subsequently induced. At arbitrary stages of reduction the grafts were retransplanted, homoioplastically, to the backs of non-metamorphosing larvæ and allowed to regenerate. The median graft was the first to complete regeneration, as determined by histological reconstitution; the ventral being the second and the dorsal third.

3. The histolysis and regeneration of tail-skin always involves an orderly sequence of cellular changes. For both of these processes the sequence was determined as epidermis-dermis-subcutis.

4. It is concluded that the histolytic sequence is due largely to a time differential in the onset of histolysis. The regenerative sequence is probably the result of a time differential in regenerative incipience.

5. The physiological basis of these sequences is inherent in the tissues, and it is inferred that they may be determined or conditioned by different metabolic rates typical of each particular level.

LITERATURE CITED

BARFURTH, D., 1887. Die Rückbildung des Froschlarvenschwanzes und die sogenannten Sarcoplasten. *Arch. f. mikr. Anat.*, **29:** 35.

BARFURTH, D., 1894. Die experimentelle Regeneration überschüssiger Gliedmassentheile (Polydaktylie) bei den Amphibien. *Arch. f. entw.-mech.*, **1:** 91.

BATAILLON, E., 1891. Récherches anatomiques et expérimentales sur la métamorphose de Amphibiens anoures. *Ann. de l'Université de Lyons*, **2:** 1.

BRADLEY, H. C., 1922. Autolysis and Atrophy. *Physiol. Rev.*, **2:** 415.

CLAUSEN, H. J., 1930. Rate of Histolysis of Anuran Tail Skin and Muscle during Metamorphosis. *Biol. Bull.*, **59:** 199.

CLAUSEN, H. J., 1932. Rate of Regeneration of Partly Histolyzed Anuran Tail Skin. *Biol. Bull.*, **63:** 129.

COLE, W. H., 1922. The Transplantation of Skin in Frog Tadpoles with Special Reference to the Adjustment of Grafts over Eyes, and to the Local Specificity of Integument. *Jour. Exper. Zool.*, **35:** 353.

DURBIN, M. L., 1909. An Analysis of the Rate of Regeneration throughout the Regenerative Process. *Jour. Exper. Zool.*, **7:** 397.

ELLIS, M. M., 1908. Some Notes on the Factors Controlling the Rate of Regeneration in Tadpoles of Rana clamata—Daudin. *Biol. Bull.*, **14:** 281.

ELLIS, M. M., 1909. The Relation of the Amount of Tail Regenerated to the Amount Removed in Tadpoles of Rana clamitans. *Jour. Exper. Zool.*, **7:** 421.

HELFF, O. M., 1926. Studies on Amphibian Metamorphosis. I. Formation of the Opercular Leg Perforation in Anuran Larvæ during Metamorphosis. *Jour. Exper. Zool.*, **45:** 1.

HELFF, O. M., 1930. Studies on Amphibian Metamorphosis. VIII. The Rôle of the Urostyle in the Atrophy of the Tail. *Anat. Rec.*, **47:** 177.

HELFF, O. M., 1932*a*. Studies on Amphibian Metamorphosis. X. Hydrogen-ion Concentration of the Blood of Anuran Larvæ during Involution. *Biol. Bull.*, **53:** 405.

HELFF, O. M., 1932*b*. Differences in Electrical Potential between Various Levels of the Tail of Anuran Larvæ. *Anat. Rec.*, **54:** 67 (abstract).

HELFF, O. M., AND H. J. CLAUSEN, 1929. Studies on Amphibian Metamorphosis. V. The atrophy of anuran tail muscle during metamorphosis. *Physiol. Zool.*, **2:** 575.

HERRICK, E. H., 1932. Mechanism of Movement of Epidermis, Especially its Melanophores, in Wound Healing, and Behavior of Skin Grafts in Frog Tadpoles. *Biol. Bull.*, **63:** 271.

LINDEMAN, V. E., 1929. Integumentary Pigmentation in the Frog, Rana pipiens, during Metamorphosis, with Especial Reference to Tail-Skin Histolysis. *Physiol. Zool.*, **2:** 255.

MERCIER, L., 1906. Les processus phagocytaires pendant la métamorphose des Batraciens anoures et des Insectes. *Arch. d. zoöl. expér. et gén.*, **5:** 1.

MORGAN, T. H., 1906. The Physiology of Regeneration. *Jour. Exper. Zool.*, **3:** 457.

MORSE, W., 1918. Factors Involved in the Atrophy of the Organs of the Larval Frog. *Biol. Bull.*, **34:** 149.

SAGUCHI, S., 1913. Über Mitochondrien (Chondriokonten) und mitochondriale Stränge (Sog. Eberthsche intrazelluläre Gebilde) in den Epidermiszellen der Anurenlarven nebst Bemerkungen über die Frage der Epidermis-Cutisgrenze. *Arch. f. mikr. Anat.*, **83:** 177.

SCHUBERT, M., 1926. Untersuchungen über die Wechselbeziehungen zwischen wachsenden und reducktiven Geweben. *Zeitschr. f. mikr. Anat. Forschung.*, **6:** 162.

SPALLANZANI, A. L., 1769. Prodomo, An Essay on Animal Reproduction. Trans. by M. Maty, London.

SPEIDEL, C. C., 1926. Studies of Hyperthyroidism. IV. The Behavior of the Epidermal Mitochondria and the Pigment in Frog Tadpoles under Conditions of Thyroid-accelerated Metamorphosis and of Regeneration following Wound Infliction. *Jour. Morph. Physiol.*, **43**: 57.

ZELENY, C., 1916. Studies on the Factors Controlling the Rate of Regeneration. *Ill. Biol. Mon.*, **3**: 1.

THE MOVEMENT AND REGULATION OF CILIA ON THE PARAPODIA OF NEPHTHYS BUCERA, EHLERS [1]

B. R. COONFIELD

(From the Department of Biology, Brooklyn College, and the Marine Biological Laboratory, Woods Hole, Mass.)

The mechanism concerned with ciliary control has been studied extensively, from both the morphological and physiological features of this effector system. Included in the physiological aspects of these studies are such phases as ciliary movement and ciliary coördination. Even though numerous publications concerned with cilia have appeared, we are still uncertain as to the exact mechanism responsible for ciliary movement and of the principle involved in ciliary coördination. Perhaps the smallness of cilia and the fact that they break up so readily during either chemical or mechanical treatment are problems preventing a thorough understanding of this type of effector system.

The cilia on the body wall and on the branchiae of *Nephthys bucera* have been used in the experiments reported here because their size is unusually large and they appear on structures in convenient locations for study. Since *Nephthys bucera* has not been studied in detail, even by Ehlers (1868), who described the type specimen and who made no mention of any ciliary organs, a part of this report deals with the structure of these cilia-bearing regions. Consideration also is given here to the problem of ciliary coördination.

The distribution and function of cilia on the parapodia of *Nephthys bucera,* together with the direction of their effective and metachronal waves, have been described by Coonfield (1931). These cilia are located on the branchia of each notopodium and they continue in a single line on the body wall to the base of the neuropodium. They are definitely grouped into small tufts, each of which is limited to a single cell (Figs. 5, 7, and 8). There are 18 or more of these tufts on the outer and on the inner margin of each branchia and 20 or more on the body wall between the two parts of a parapodium. Each ciliary tuft is made up of 40 to 50 large compound cilia that vary in length, the shorter ones being at the margins of each tuft. Also the tufts decrease in length gradually from the longest ones at the base of the branchia to the shortest one on its tip. The cilia on the body wall are slightly longer

[1] Contribution No. 14 from the Department of Biology, Brooklyn College. This work was begun at the Zoölogical Laboratories, Harvard University.

than those on the branchiae. The longest cilia measured were about 62 μ while the shortest ones were 5.6 μ. Gray (1928) has stated that cilia vary from 0.1 μ–0.3 μ in diameter and are often 15 μ in length. This author is obviously including only the simple type of cilia, for Carter (1924) and Bhatia (1926) have given 30 μ for the length of the complex latero-frontal cilia of *Mytilus*. That these large cilia of *Nephthys* are of the compound type is shown definitely by their breaking up into smaller units when treated with fixing reagents and when a ciliated cell is disintegrating after having been punctured. This breaking up into smaller units begins at the tip of the cilium and progresses to its base. In that these cilia are compound, they are similar to those of the nudibranch veliger (Carter, 1926) and to the latero-frontal cilia of *Mytilus* (Carter, 1924). Although the ciliary units could be seen, it was not possible to determine their exact form.

According to Gray (1928), there are three types of ciliary movement: the pendular type which includes only those cilia that vibrate backwards and forwards by flexure at their bases; the flexural type composed only of cilia that move by a flexure beginning at their tips and passing down to their bases, thereby bending the cilia into hook-like structures; and the undulatory type which is characterized by a series of waves passing from the base of the cilium to its tip. The cilia of *Nephthys bucera* move according to the flexural type and thus resemble those of the gills of *Mytilus*. The cilia of *Mytilus* (Gray, 1920) are stiff in their effective beat and are limp in their return stroke. The cilia of *Nephthys* are not quite as flexible in their recovery beat as are those of *Mytilus* and they come to rest at the end of the effective beat rather than at its beginning.

The metachronal wave of the cilia of *Nephthys* as it moves from the base of the outer edge of the branchia on around the tip and along the body wall shows clearly a rhythmic movement. The metachronism is produced by simultaneous beating of the cilia at corresponding edges of each tuft, and thus moves along the line of these tufts in a perfectly synchronized pattern. This wave moves in a clockwise direction, passing towards the neuropodium on the right side and away from the neuropodium on the left side of the animal (Coonfield, 1931). It is interesting to note that this direction of ciliary movement corresponds to that of the prototrochal cilia of *Spirobranchus tricornis* and *Eunice fucata* as reported by Mayer (1910). Since the direction of the effective stroke and that of the metachronal wave of cilia on animals are in most cases either the same or directly opposite to each other, it is of further interest to note that these directions of movement in *Nephthys* are at right angles to each other. The cilia of this worm at correspond-

ing regions on the opposite margins of a branchia reach the opposing phases of their movement at the same time. Thus when a cilium on one side of the branchia reaches the end of its effective stroke, the corresponding one opposite is at the end of the recovery stroke (Fig. 1). This form of beating produces a current which carries suspended particles in a circular fashion around an isolated branchia. The effect of ciliary movement on the body wall, however, combines with that of the

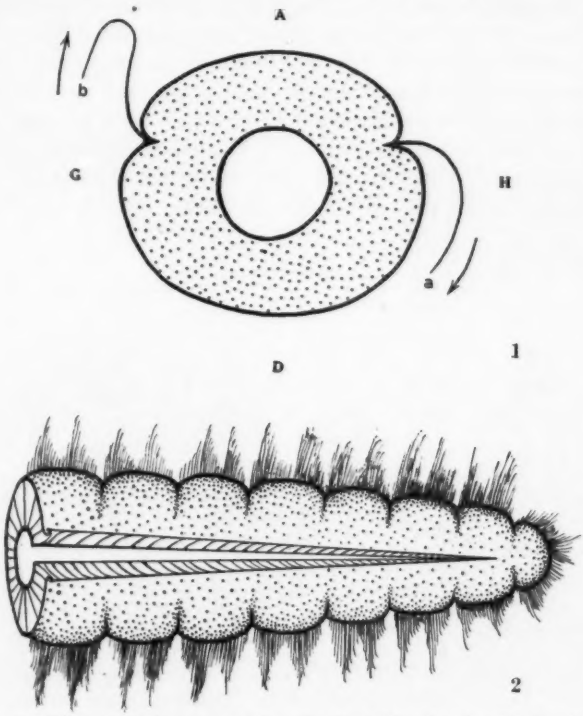


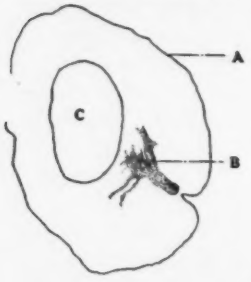
FIG. 1. Diagrammatic cross-section of a branchia, showing the location of its cilia with the relationship of their phases of movement. *A*, anterior; *D*, posterior; *G*, inner margin; *H*, outer margin; *a*, end of effective beat; *b*, end of recovery stroke.

FIG. 2. Diagrammatic view of a branchia, showing the cut made in testing for the ciliary coördinating system.

branchial cilia to carry liquids and other materials posteriorly along the body of the worm. This movement of water over the branchiae of *Nephthys* is a definite aid to respiration. This is of significance since this animal usually lives in about six inches of sand and does not possess any other accessory respiratory organs.

Structures in the Ciliated Region as Shown by Stains

Iron hematoxylin, with or without a counter stain, showed a tuft of cilia to be limited to a single cell. Each of these cells possesses a single nucleus. This condition is quite unlike the syncytial form as reported for the latero-frontal epithelium of *Lampsilis* by Grave and Schmitt (1925). These ciliated cells of *Nephthys* are distinctly separate. Small intracellular fibrils which pass from the base of each cell to its cilia could be seen (Figs. 7 and 8). There was no evidence of a cone-shaped fibrous structure as found by Grave and Schmitt (1925) in the latero-frontal epithelium of the gill of *Lampsilis* nor was there any degree of association between these fibers and the cell nucleus. Iron hematoxylin did not show any basal corpuscles associated with the cilia, as reported by Saguchi (1917) for ciliated epithelium of *Anodonta*. Many fibers were shown by this stain within the basal membrane beneath these ciliated cells. There was no definite morphological associa-



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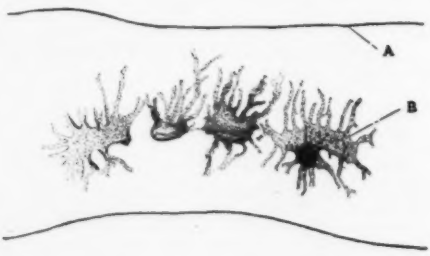

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Figs. 3 and 4. Camera-lucida drawings of ciliary regions to show the effect of methylene blue on ciliated cells. The drawings are of the cross-section and sagittal section respectively. *a*, branchial wall; *b*, intracellular stained materials; *c*, branchial cavity. (Methylene blue 1½ hours, 7 μ, × 440.)

tion, however, between these fibers and those of the cilia even though these fibers were near each other. Vom Rath's stain showed these cells as having a granular cytoplasm rather than a fibrous one as was shown by iron hematoxylin (Fig. 9). Immersion of pieces of a branchia or even whole segments of *Nephthys* in very dilute solutions of methylene blue from 1 to 8 hours produced effects on the cytoplasm of the ciliated cells similar to that of Vom Rath's stain, except that in methylene blue the cytoplasm stained more deeply in the central part of the cell near the nucleus and showed the root-like substance converging toward the nucleus (Figs. 3, 4 and 6). If, however, the tissue remained in methylene blue 8 hours or longer the stained substance was shown broken up near the basal membrane (Fig. 6). Neither basal corpuscles nor ciliary rootlets were shown by methylene blue.

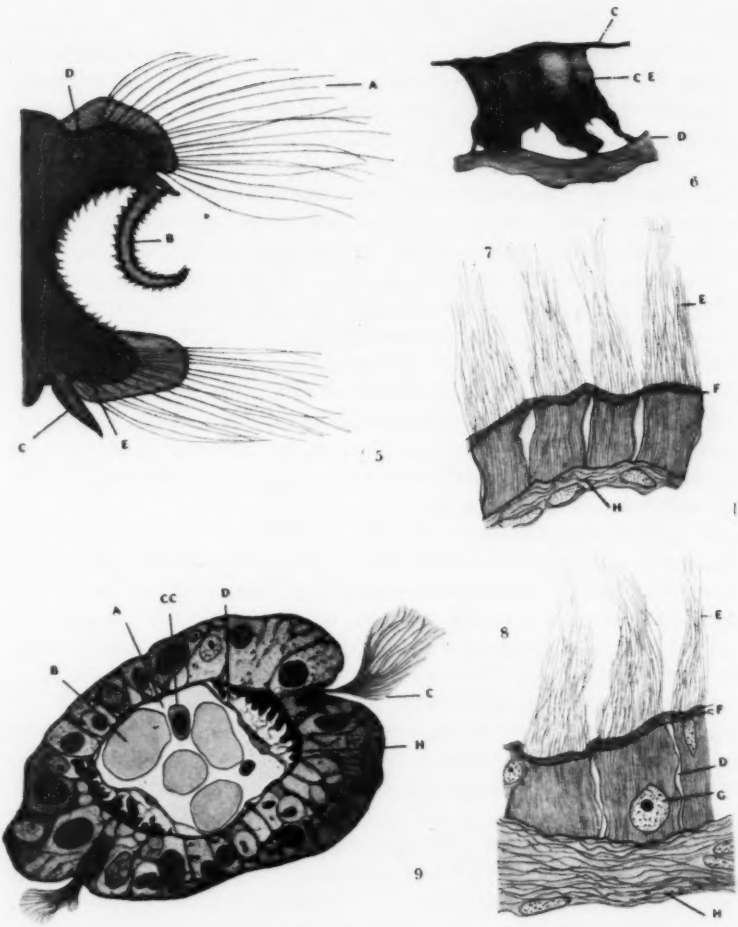

FIG. 5. Camera-lucida drawing of a parapodium of the right side of *Nephthys*. *A*, setæ; *B*, branchia; *C*, ventral cirrus; *D*, notopodium; *E*, neuropodium.

FIG. 6. Camera-lucida drawing of a ciliated cell of *Nephthys* to show the effect of staining with methylene blue at 8 hours. *C*, cuticula; *CE*, cell structure; *D*, basal membrane. (7 μ, $\times$ 900.)

FIGS. 7 and 8. Camera-lucida drawings of ciliary regions to show intracellular materials stained in iron hematoxylin, just to one side of the nucleus and through the nucleus respectively. *D*, intercellular space; *E*, cilia; *F*, cuticula; *G*, nucleus; *H*, basal membrane. (10 μ, $\times$ 440.)

FIG. 9. Camera-lucida drawing of a cross-section of a branchia, showing the structure of this organ; *A*, branchial cavity; *B*, blood vessel; *C*, cilia broken into their units; *CC*, cœlomic corpuscle; *D*, peritoneum; and *H*, branchial muscles. (Vom Rath, 6 μ, $\times$ 900.)

Reactions of Cilia to Physiological Tests

The ciliated regions of *Nephthys* are in convenient locations for employing physiological methods in testing for the ciliary regulatory system. Either pieces of a branchia or a whole branchia isolated from an animal's body will live for several hours without either the rhythm or the amplitude of their cilia being changed from the normal. A single isolated branchia was slit from end to end on one side between the opposite rows of cilia (Fig. 2). The beating of its cilia with their metachronal wave continued in the regular manner after this cut was made. Within a few hours, however, this tissue disintegrated, but the ciliary beating was normal until the beginning of disintegration.

Pieces of branchiae were impaled on a cover slip by means of a micro-needle and the ciliated cells were treated in the following ways: 1. A single ciliated cell was punctured with a micro-needle and it broke up immediately. Its cilia stopped beating as soon as this dissipation reached them and thus confirmed the views of Chambers (1924). 2. A cell bearing active cilia, intact with other similar cells, was pressed near its base. Its cilia became quiescent as this pressure was applied, but the cilia of the adjoining cells continued their regular beating. The quiescent cilia regained their beating and rhythm upon the release of this pressure. 3. A single active ciliary cell between two other similar ones was destroyed by the puncture method. The cilia on this cell ceased beating while those on the other cells did not change their rate or their metachronal movement. 4. Pressure was applied on non-ciliated cells adjoining active ciliated ones without affecting these active cilia. 5. One ciliated cell was pressed at the bases of its cilia near the center of the tuft. The cilia in the pressed region stopped beating. Those on either side of the quiescent cilia continued to beat, but with a slower rate. As soon as the pressure was released, however, all of the cilia resumed their normal beating. 6. A group of ciliated cells was stretched by a pull with microneedles. Their cilia became quiescent, but resumed normal movement as soon as the stretching was discontinued. 7. A few cilia in a single tuft were held for a few seconds. The other cilia in this tuft continued to beat without changing either their rate or their metachronism.

Discussion

The beating of cilia serves the animals on which they are located in one of two specific ways. In the one, they beat continuously in an automatic manner and move substances along a surface. Though the activity of these cilia is thought by some to be independent of other tissues

and therefore not controlled by the nervous system, evidence has been presented by Agersborg (1923), Albertoni (1891), McDonald (1927), Merton (1923), Meyer (1882), Seo (1931), and Setna (1930) which shows that certain of these non-locomoter type of cilia are nervously controlled. That such cilia on some animals are nervously independent, however, is strongly indicated by the continued beating of cilia for several hours on small isolated pieces of ctenophores, mollusks, and annelids. The other function of cilia is to serve as locomotor structures. These cilia may serve as the entire locomotor system or they may act only as an aid to another type of effector system during locomotion. Since the cilia in this category must change either their rate or direction of beating as the animal changes its rate or direction of movement or must cease beating entirely, it has been assumed that they are under the control of nerves. Certain data of experimental and structural origin have been given to demonstrate the view of nervous control (Copeland, 1922; Carter, 1926). It has been suggested that these nerves are of the inhibitory type. If this be true, then it is natural to assume that the state of ciliary activity is the normal condition of ciliary protoplasm. This view is supported by Chambers (1924) who, in referring to observations made on the ciliated cells of the ovaries of sea urchins, stated (p. 258), " An active cell can be impaled on the end of a needle without interfering with the movements of the cilia. If, however, the surface of the cell at its base be pricked and ever so slightly torn, a breakdown travels rapidly over the cell. As the injury reaches the ciliated border the peripheral cilia stop beating and the further advance of this injury can be followed by the successive cessation of more and more cilia until all ciliary activity has ceased. The cytoplasm then becomes dissipated in the sea water and the cilia fall apart as motionless filaments."

That certain stains show nerves and fibers within ciliated cells in various animals is clearly stated in reports of several investigators. Meyer (1882) found nerve fibers ending in a layer of ganglion cells below the epithelium of *Polyophthalmus*. Agersborg (1923) noted neural fibers in *Melibe* extending from the pedal ganglion to the bases of ciliated, epithelial cells where these fibers joined the intracellular fibers that were connected to the ciliary basal bodies. Carter (1926) found nerve fibers passing up between the bases of the ciliated cells of the nudibranch veliger, and later (1928) he showed nerve fibrils passing from the cerebral ganglion and terminating in the ciliated cells of this veliger. He found also intracellular fibers passing from these nerve fibrils to the basal granules within the ciliated cells. Setna (1930), however, could not find definite nervous connections in the

ciliated cells of *Pecten*, and Bhatia (1926), even though he found intercellular fibrils in ciliated epithelium of *Mytilus* connecting to the subepithelium, concluded that coördination of movement is effected by cell contact rather than by any specialized fibrillar mechanism. Although fibrils within the ciliated cells and fibers within the basal membrane were seen in the stained tissues of *Nephthys* (Figs. 7 and 8), there was no evidence of connection between these two sets of fibers. Even if these stained materials represent nervous elements, physiological evidence must support this view before we can be certain that the nervous system really regulates ciliary activity. That stained fibers may not be true nervous elements has been shown by Carter (1924), who concluded that the fibers of the lateral cells of *Mytilus* brought out by stains do not represent the nature of true fibers in the living cell.

Certain physiological experiments on ciliated tissue do support the view of ciliary coördination by nerves. Copeland (1922), by cutting ciliated epithelium of the foot of *Polinices*, demonstrated that impulses were carried from the subepithelial tissue to these ciliated cells. Merton (1923) produced activation of quiescent cilia on the epithelium of the snail by stimulating electrically the nerves leading to this tissue. McDonald (1927) found that the pharyngeal cilia of the frog beat more rapidly when their sympathetic nerve supply was stimulated and they beat less rapidly when their parasympathetic nerves were stimulated. Seo (1931) reported that ciliary movement in the palate of the frog and the toad were reflexly accelerated by the stimulation of the tongue or the glossopharyngeal nerves. Although these experiments do show certain facts which indicate the nervous regulation of ciliary beating, the continued rhythmic movement of cilia on isolated pieces of tissues shows automatic activity which is indicative of nervous independence. This lack of nervous regulation was demonstrated on *Nephthys* when the destruction of a ciliated cell between two other similar cells failed to interfere with the beating of their cilia. It might be said that the regulating impulses lie in the basal membrane, where definite fibers were found, and therefore the destruction of a single ciliated cell does not affect this transmitting system. Since the application of pressure, however, to the adjoining basal membrane cells failed to interrupt ciliary movement, whereas the application of the same amount of pressure to the ciliated cells did stop their activity, it is assumed that the basal membrane is not concerned in the regulation of these cilia. The results of experiments on the ciliated cells of *Nephthys* show them to be independent of any nervous control. This is in agreement with the results of Lucas (1931*a*), who stated that the ciliated epithelium of *Mytilus* is not regulated by a nervous system. Lucas (1931*b*) also

cut between two active latero-frontal ciliated cells of a lamellibranch gill without producing any effect on the movement of their cilia. Furthermore, Gray (1922) came to the conclusion that the cilia of *Mytilus* are entirely independent of any nervous control.

Lucas (1932*a*) concluded from results of experiments on the latero-frontal cell of *Modiolus* that an impulse sufficient to stimulate other quiescent cilia to activity is transmissible through cells bearing quiescent cilia. This cannot be the entire activating mechanism in *Nephthys* since a cell between two active ciliated cells was destroyed and yet the cilia of these two cells continued to beat normally. Not only is the ciliary movement of *Nephthys* independent of the nervous system but the coördination of the metachronal wave is independent of this system also. Since the evidence shows the movement and the metachronal wave of cilia of *Nephthys* to be independent of the nervous system and since these cilia continue to beat in a normal manner on an isolated cell, then each cell must possess its own regulating mechanism. This view has been expressed by Worley (1933), who believes that the individual cell governs its own metachronism. Possibly a neuroid mechanism is concerned with the regulation of cilia when they are in their normal location on the animal, but this type of system cannot account for the entire coördination. The type of apparent independence of the cilia of *Nephthys* is not unusual since Gray (1924) has drawn a parallel to this in the contraction of cardiac muscle.

From the data given by others in regard to the control of various ciliated tissues and from the results of experiments on the ciliary system of *Nephthys*, it is apparent that no general statement in regard to ciliary control can be made with any degree of exactness. This controlling system is most likely different for different ciliated materials.

Summary

1. The cilia of *Nephthys* are grouped into small tufts on the branchia and on the body wall of each parapodium. Each tuft is limited to a single cell and consists of 40 to 50 large compound cilia. Each compound cilium is made up of smaller units.

2. The longest cilia measured were 62 μ while the shortest ones were 5.6 μ.

3. The cilia of *Nephthys* in their effective and recovery strokes move according to the flexural type.

4. The corresponding cilia of each tuft along the branchia move synchronously while those exactly across the branchia are opposite in their phases of movement.

5. The effective stroke of the cilia is at right angles to the metachronal wave.

6. Iron hematoxylin, with or without a counter stain, showed intracellular fibrils passing from the base of each ciliated cell to the cilia. This stain showed also many fibers within the basal membrane beneath the ciliated cells. There was no connection between the fibers within the basal membrane and the intracellular fibrils of the ciliated cells.

7. Both Vom Rath's stain and methylene blue showed the cytoplasm of the ciliated cells to be granular.

8. Physiological tests indicate each ciliated cell to be independent and that it regulates its own activity.

9. Stains and the results of physiological experiments indicate the ciliary system of *Nephthys* to be independent of the nervous system. Possibly a neuroid mechanism is responsible for the ciliary coördination of these cells when they are in their natural location.

LITERATURE CITED

AGERSBORG, H. P. K., 1923. The Morphology of the Nudibranchiate Mollusc Melibe (syn. Chioræra) leonina (Gould). *Quart. Jour. Micr. Sci.*, **67**: 507.

ALBERTONI, P., 1891. Wirkung des Cocains auf die Contractilität des Protoplasma. *Pflüger's Arch.*, **48**: 307.

BHATIA, D., 1926. The Structure of the Latero-frontal Cells of the Gills of Mytilus edulis. *Quart. Jour. Micr. Sci.*, **70**: 681.

CARTER, G. S., 1924. On the Structure and Movements of the Latero-frontal Cilia of the Gills of Mytilus. *Proc. Roy. Soc.*, Ser. B, **96**: 115.

CARTER, G. S., 1926. On the Nervous Control of the Velar Cilia of the Nudibranch Veliger. *Brit. Jour. Exper. Biol.*, **4**: 1.

CARTER, G. S., 1928. On the Structure of the Cells Bearing the Velar Cilia of the Nudibranch Veliger. *Brit. Jour. Exper. Biol.*, **6**: 97.

CHAMBERS, R., 1924. The Physical Structure of Protoplasm as Determined by Micro-dissection and Injection. Chapter 5, p. 258. Cowdry's General Cytology, The University of Chicago Press.

COONFIELD, B. R., 1931. The Cilia of Nephthys bucera. *Proc. Nat. Acad. Sci.*, **17**: 416.

COPELAND, M., 1922. Ciliary and Muscular Locomotion in the Gastropod Genus Polinices. *Biol. Bull.*, **42**: 132.

EHLERS, E., 1868. Die Borstenwürmer (Annelida Chætopoda). Abt. 1–2, Leipzig.

GRAVE, C., AND F. O. SCHMITT, 1925. A Mechanism for the Coördination and Regulation of Ciliary Movement as Revealed by Microdissection and Cytological Studies of Ciliated Cells of Mollusks. *Jour. Morph.*, **40**: 479.

GRAY, J., 1920. The Effects of Ions upon Ciliary Movement. *Quart. Jour. Micr. Sci.*, **64**: 345.

GRAY, J., 1922. The Mechanism of Ciliary Movement. *Proc. Roy. Soc.*, Ser. B, **93**: 104.

GRAY, J., 1924. The Mechanism of Ciliary Movement. IV. The Relation of Ciliary Activity to Oxygen Consumption. *Proc. Roy. Soc.*, Ser. B, **96**: 95.

GRAY, J., 1928. Ciliary Movement. The MacMillan Company, N. Y.

LUCAS, A. M., 1931*a*. The Distribution of the Branchial Nerve in Mytilus edulis and its Relation to the Problem of Nervous Control of Ciliary Activity. *Jour. Morph. and Physiol.*, **51**: 195.

LUCAS, A. M., 1931*b*. An Investigation of the Nervous System as a Possible Factor in the Regulation of Ciliary Activity of the Lamellibranch Gill. *Jour. Morph. and Physiol.*, **51**: 147.

LUCAS, A. M., 1932*a*. Coördination of Ciliary Movement. I. Methods of Study and the Relation of Ciliary Coördination to Ciliary Inhibition. *Jour. Morph.*, **53**: 243.

LUCAS, A. M., 1932*b*. Coördination of Ciliary Movement. II. The Effect of Temperature upon the Ciliary Wave Length. *Jour. Morph.*, **53**: 265.

MCDONALD, J. F., C. E. LEISURE, AND E. E. LENNEMAN, 1927. Neural and Chemical Control of Ciliated Epithelium. *Proc. Soc. Exper. Biol. and Med.*, **24**: 968.

MAYER, A. G., 1910. I. The Converse Relation Between Ciliary and Neuromuscular Movements. *Carnegie Inst. Wash.*, Publ. 132, p. 1.

MERTON, H., 1923. Studien über Flimmerbewegung. *Pflüger's Arch.*, **198**: 1.

MEYER, E., 1882. Zur Anatomie und Histologie von Polyophthalmus pictus Clap. *Arch. f. Mikr. Anat.*, **21**: 769.

SAGUCHI, S., 1917. Studies on Ciliated Cells. *Jour. Morph.*, **29**: 217.

SEO, A., 1931. Studies on the Nervous Regulation of the Ciliary Movement. *Jap. Jour. Med. Sci., III. Biophysics*, **2**: 47.

SETNA, S. B., 1930. The Neuro-muscular Mechanism of the Gill of Pecten. *Quart. Jour. Micr. Sci.*, **73**: 365.

WORLEY, L. G., 1933. Metachronism in Ciliated Epithelium. *Proc. Nat. Acad. Sci.*, **19**: 320.

FACTORS CONTROLLING LONGEVITY IN PROTOZOAN PROTOPLASM

GARY N. CALKINS

(From the Department of Zoölogy, Columbia University, New York City)

I well remember the thrill I got when I first saw a living *Amœba proteus* under the microscope; its glairy substance, evidently liquid but not mixing with the surrounding water, its enigmatic movements and method of feeding were all fascinating. Nor was the thrill lessened in any degree when I realized that this bit of living protoplasm, in all probability, had been continuously living since the beginnings of life and, barring accidents, had the possibility of an indefinitely continued existence in the future.

What is true of *Amœba* is equally true of the many thousands of species of other Protozoa, some of which are vastly more complicated. The protoplasm composing each of them and each with its specific make-up or organization has been continuously living in the past and has the possibility of living continuously in the future.

What are the factors underlying this remarkable longevity, and what are the observable processes by which this marvellous continuity is maintained? In the following pages I shall attempt to answer these questions.

Weismann, more than fifty years ago in his classical essays on "Life and Death," dealt with this problem in his characteristically suggestive way. Protozoa, he argued, are simple bits of protoplasm which in a proper medium have within themselves all that is necessary for an indefinitely continued life. They receive stimuli and food from the environment, react to the stimuli, ingest and digest the food and grow. When they reach the limit of growth typical of the species, they divide. A fully grown bit of protoplasm does not die but its living substance is parcelled out to two daughter cells each of which repeats the cycle of feeding, growth, and division. Natural death, therefore, in Protozoa, is unknown—there is no corpse.

Protozoa, he continued, are equivalent to the germ cells of Metazoa which are, likewise, potentially immortal. But germ cells upon development give rise to specialized cells, tissues, and organs which have a limited potential of vitality and these ultimately die. Natural death, said Weismann, is the penalty which Metazoa pay for the privilege of specialization.

Weismann, in common with his contemporaries, recognized what we regard as the first or primary group of factors underlying longevity, viz. a proper medium, and the fundamental metabolic activities of the organism; life without these is unthinkable. His other conclusions, with our knowledge today of Protozoa, are not acceptable. Metazoa and Protozoa are not fundamentally different but are fundamentally alike, with differences only in degree.

Fundamental and Derived Organization

The egg cell of a metazoön is a small, apparently homogeneous sphere of protoplasm which will develop into exactly one type of organism. It has a specific make-up or fundamental organization which differs from the fundamental organization of every other type of egg cell. Upon stimulation it develops by continued cell division into organs and structures of a specific type of adult with a characteristic derived organization which is the basis of classification. The derived structures constitute the soma and together they protect and nourish the germ plasm with its fundamental organization within. Upon exhaustion of the potential of vitality of the derived organization the individual dies a natural death and its germ plasm, without protection and nourishment, is killed. The derived organization in the vast majority of Metazoa is not turned back or resolved again into the germ plasm with its fundamental organization.

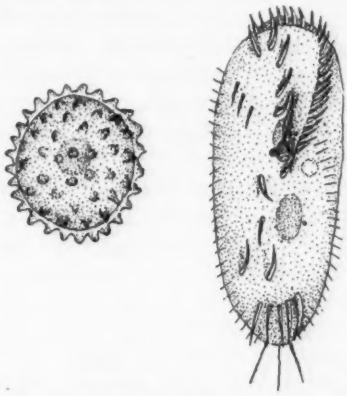

Fig. 1. *Stylonychia pustulata.* Encysted organism with its fundamental organization, and the adult organism with its derived organization.

Let us now look at a protozoön, preferably a ciliate with its fairly complex organization. Like most ciliates, it may be found in an active vegetative, or in an encysted, quiescent condition. The cyst of any species of the genus *Stylonychia* is a small spherical structure with a characteristic corrugated external surface (Fig. 1). Within it is a ball of

apparently homogeneous and structureless protoplasm. Like an egg cell, it has a specific fundamental organization which will develop into one specific type of adult and into nothing else. Place such a cyst in a suitable nutrient medium; soon the cyst wall softens and becomes permeable to water and oxygen and the fundamental organization develops quickly into an active organism with its characteristic adult derived organization (Fig. 1). Unlike a metazoön, the derived structures are not formed by cell division but are differentiated parts of the living fundamental organization itself, just as an egg cell might bud out derived structures continuous with its own protoplasmic make-up. Like the soma of a metazoön, these derived structures are vitally important to the organism in its metabolic activities. In *Stylonychia* they consist of macronuclei, mouth gullet and gastric vacuoles, peristome, anal opening, undulating membranes, membranelles and various types of cirri, contractile vacuole and canals. They are formed from and are continuous with the fundamental organization.

In different types of Protozoa derived organizations have a widely varied significance both as to origin and as to function. With Doflein we may group them in two categories—the euplasmatic and the alloplasmatic. The former are differentiations of the living protoplasm such as we find in pseudopodia of the Sarcodina, epimerites of the Gregarinida, or in the various motile and cortical organs of the Infusoria. The latter are usually products of secretion or of precipitation by the living protoplasm and are lifeless matter as in shells, tests and skeletons of Foraminifera, Radiolaria, etc., or as in gelatinous secretions (pseudochitin or tectin of Bresslau) forming houses, cups, stalks, etc. of flagellates and Infusoria. Such alloplasmatic structures of Protozoa are equivalent to the skeletal elements of the Metazoa soma and, like them, when once formed, are irreversible. With them we have justification for challenging Weismann's denial of a corpse in Protozoa. Forming an important part of the soma, a shell of a foraminiferan or a skeleton of a radiolarian is left behind with a small bit of residual protoplasm when the fundamental organization breaks up into a multitude of germ cells which leave the old cell behind as a relic of the old generation. The sea bottom is covered fathoms deep with such corpses of previously living individuals.

The euplasmatic structures of the derived organization, on the other hand, have no such fate. Formed by and from the fundamental organization, they have a limited potential of functional activity, then are withdrawn or absorbed into the fundamental organization and new structures are formed to replace them. This phenomenon of absorption and replacement termed *reorganization* occurs at critical periods in the life history and, with its different manifestations, constitutes the second

and in some ways the most important group of factors underlying longevity.

The fundamental organization of a ciliate is undoubtedly found in its purest form in the permanent cyst. Prior to encystment the motile organs and other cortical differentiations are absorbed; the macronucleus fragments and the fragments are absorbed; water and waste matters in the endoplasm are discarded and an impervious covering membrane, the cyst wall, is secreted from the peripheral zone of protoplasm. The micronucleus divides and one of the products will form a new macronucleus. The fundamental organization, reduced to its lowest terms, devoid of water and protected by its cyst, may be dried and kept in the dried state for months or even years and without impairment of its potential vitality. The specific organization, presumably made up of nucleoproteins, proteins, carbohydrates, fats, and salts, in a definite combination, remains intact.

Ultimately, in a favorable environment, water and oxygen are introduced through the now permeable cyst wall and metabolic activities begin. Through these, structures are developed which were not present before. These are formed mainly in the cortex but the new macronucleus develops rapidly in the endoplasm. The young organism, after whirling around in its cyst, finally emerges and begins a new life cycle with high vitality. With its full complement of cell organs the physiological activities of the young organism begin at once. Reactions to stimuli, movement, ingestion of food, secretion of digestive fluids, digestion and assimilation of food, defecation of undigested remains, excretion of metabolic waste, and a multitude of other internal reactions, all go on in full swing. The organism grows.

The varied activities do not leave the protoplasm as it was when it left the cyst. Chemical processes of metabolism result in substances not present before and, transient or stable, they play their part in further activities. Physically, changes in phase in the polyphasic system are probable although not demonstrated. In short, the fundamental organization is constantly changing and is never twice the same in an active organism. Furthermore, the changes are cumulative and finally a condition is set up when some drastic reaction is essential for continued life. Reorganization, or a return of the protoplasm to the fundamental organization, now occurs and is consummated by the phenomena of cell division.

Reorganization by Division

The changing make-up of the protoplasmic organization as a result of continued metabolism from the end of one division to the advent of the next is difficult to demonstrate on any morphological basis. Physio-

logically, however, it is possible to show that the organization is not equally responsive at all stages between divisions and the implication is that changes do take place. A simple merotomy experiment illustrates this (Calkins, 1911). A marine ciliate, *Uronychia transfuga,* is cut transversely through the center so that half of the macronucleus and the micronucleus are in one fragment, the other half of the macronucleus and no micronucleus in the other fragment. If the operation is made on an individual three to five hours after its last division, the fragment with a micronucleus regenerates perfectly but the fragment without a micronucleus, while it may live for four or five days, never regenerates the missing structures. The same result is obtained if the organism to be cut is from ten to fifteen hours old, but if the individuals cut are older than this we get an increasing percentage of perfect regenerations of the amicronucleate fragments. If the individual is cut at the age of twenty to twenty-two hours, the complete regeneration of this fragment, although without a micronucleus, is invariable. This indicates a progressive differentiation in respect at least to the power of regeneration and to that extent a change in organization. Furthermore, if one individual is similarly cut while the two daughter cells at division are still connected, or shortly afterwards, the amicronucleate fragment will not regenerate the missing parts. This indicates that the condition which underlies the power to regenerate is lost with the processes of division and is not regained until the young cell has undergone a considerable period of normal metabolism.

It is evident that the period of full inter-divisional maturity is one of high catalytic activity shown by all parts of the organism and manifested by division of cellular elements and by other significant processes. In regeneration it is shown by the division of the basal bodies which underlie the formation of the regenerated cirri. In normal division it is manifested by remarkable reorganization processes in the macronucleus and by absorption of structures of the euplasmatic derived organization which are replaced by new ones.

Division of the cell thus is by no means a simple process but a vital phenomenon of the utmost importance and is accompanied by far-reaching changes in organization. These changes begin with the macronucleus, which is probably the most important of all the derived structures. The macronucleus arises from a product of an early division of the micronucleus at encystment or endomixis, or from the amphinucleus after conjugation. In an ex-conjugant it is not stained at first with the usual nuclear dyes but nucleic acid (chromatin) is manufactured in it and when it is ready for the first division it is filled with intensely staining, uniform granules of chromatin exactly like the macronucleus

after encystment. With the ensuing metabolic activities the homogeneous structure changes. In *Uroleptus,* where the history has been carefully followed, a different type of granule soon appears—the so-called X-granules. These do not stain with basic dyes and are hydrolyzed out with the Feulgen reaction. On the approach of division these become more numerous in each of the eight or more macronuclei. They apparently act as catalysts, for a characteristic nuclear cleft (Kernspalt) appears in each nucleus, associated with one large granule or with a group of two or more. Separated by the cleft, the larger portion of the nucleus now contains large-sized, uniform, and deeply-staining chromatin granules while the smaller portion, distal to the cleft, contains smaller and less deeply-staining granules (Fig. 2). The latter portion is cast

FIG. 2. Macronuclei of *Uroleptus halseyi.* X-granules in the eight macronuclei which act as catalysts whereby approximately one-third of each macronucleus with modified chromatin is cast off into the cytoplasm. The other parts, with re-organized chromatin, unite to form the single division nucleus (see Calkins, 1930).

off in the cytoplasm together with the nuclear cleft and any remaining X-granules. These cast-off parts disappear, evidently by absorption in the cytoplasm, while the remaining portions now unite to form at first an irregular fused mass which condenses to form the single ellipsoidal macronucleus, with deeply-staining chromatin granules, ready for division.

Analogous processes of macronuclear chromatin elimination, which I have elsewhere referred to as evidence of "nuclear purification," occur in different ways in other ciliates. Kidder (1933) described a core of modified chromatin in the center of the macronucleus of *Conchophthirius* (*Kidderia*) *mytili*. This core condenses into a small deeply-staining ball which, upon division of the macronucleus, remains for a time in the connecting strand between the daughter nuclei but ultimately disappears by absorption in the cytoplasm (Fig. 3). A similar extrusion, referred

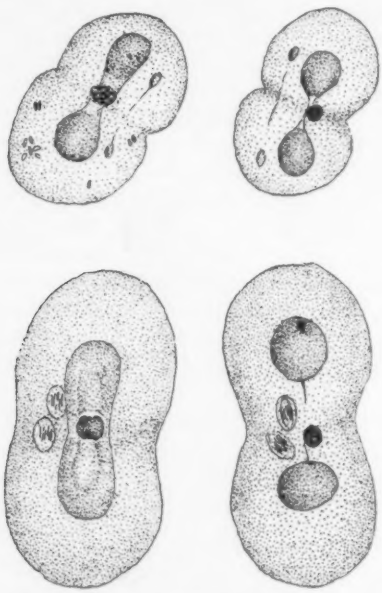

FIG. 3. *Conchophthirius anodontæ* (upper row) to show segregation and emission of the extrusion chromatin. *Kidderia* (Raabe) *mytili* (lower row) in same stages. From preparations presented by Dr. G. W. Kidder (see Kidder, 1933).

to only incidentally by Rossolimo and Jakimowitsch (1929), occurs in *Conchophthirius steenstrupii*. Here it is in the form of a finely granular substance which comes from the macronucleus and remains for a time between the nuclear halves after division but ultimately disappears in the cytoplasm.

There is reason to believe that this phenomenon has something to do with re-establishing full metabolic power of the macronuclear chromatin, possibly by the elimination of waste products of chromatin activity.

Another process of macronuclear reorganization which does not involve the actual elimination of nuclear substance occurs in the families Aspidiscidæ and Euplotidæ of the hypotrichous ciliates. At the approach of division in *Aspidisca,* according to Summers (1935), a granule appears at about the center of the convex side of the C-shaped macronucleus. The substance of the granule stretches across the macronucleus in two bands which form a V with the remains of the granule at the apex. The two arms of the V now move in opposite directions, traversing the entire macronucleus and disappearing at the two ends (Fig. 4). The increasing zone between the two arms is filled with

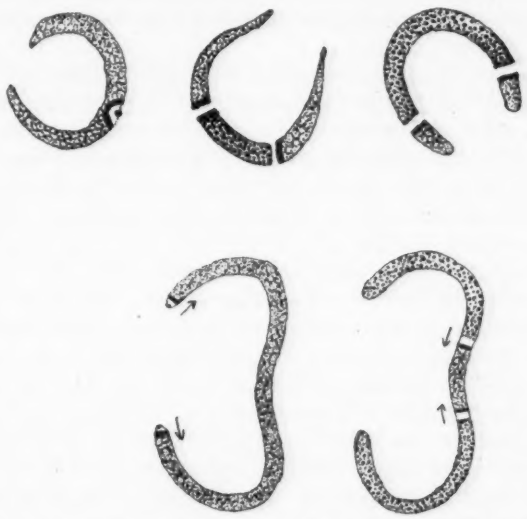

FIG. 4. *Aspidisca lynceus.* (Top row.) Macronucleus with origin of the reorganization bands in center, and two phases in their migration towards the ends. From preparations presented by Mr. F. M. Summers (see Summers, 1935).

Euplotes patella (lower row); the reorganization bands originate at the two ends of the macronucleus and pass through the nucleus until they meet in the center. Chromatin between the bands is in the form of a reticulum. This is changed into discrete granules with passage of the bands (see Turner, 1930).

deeply-staining chromatin granules which are quite different in appearance and in staining capacity from the chromatin in parts of the nucleus which have not been traversed by the bands. After disappearance of the bands at the ends, the nucleus condenses to form the typical division macronucleus of *Aspidisca.* Here again, therefore, there has been a change in the physical condition of the chromatin and a change that

is brought about through the activity of nuclear cleft substances acting as catalysts.

A similar macronuclear reorganization is brought about in *Euplotes*. Here, as a division period approaches, a nuclear cleft develops at each end of the long C-form macronucleus (Fig. 4). These two clefts now move towards each other, traversing the entire nucleus until they meet in the center where they disappear. The middle portion between the two clefts contains chromatin in the form of a reticulum, but the ends, after the clefts have passed, contain large, independent and deeply-staining chromatin granules indicating that the chromatin undergoes some kind of physical change with the passing of the bands. After the bands disappear, the nucleus shortens into a rod-shaped structure now ready for division.

This remarkable phenomenon has been described by numerous observers. Although previously observed, the clefts, or " reorganization bands " so-called by Yocom (1918), were first adequately described by Griffin (1910) in *Euplotes worcesteri*. He called them " reconstruction bands " and described them as made up of two parts, one of which, a proximal part turned towards the center of the nucleus, is deeply and uniformly stained. Griffin regarded this as a " solution plane " in which the chromatin is completely dissolved. The second portion, which he called the " reconstruction plane " is in close contact with the solution plane and in most cases appears uniformly clear and unstained and with no trace of structure. In some cases he found what appeared to be linin fibrils on the distal ends of which minute chromatin granules were seen; these enlarge rapidly into intensely staining discrete granules characteristic of the distal ends of the nucleus. Griffin believed that the old chromatin is completely dissolved in the solution plane and is reformed without the impurities of the old chromatin—a process of nuclear rejuvenescence.

Yocom (1918) gives a somewhat different interpretation of these enigmatic structures which he calls " reorganization bands " in the related species *Euplotes patella*. The two parts of each band are present as in the description above, but Yocom holds that the chromatin does not go into solution in what Griffin calls a solution plane, but " appears there as closely packed granules from which they pass over the reconstruction plane not as precipitated chromatin but as granules which have undergone some sort of physical and possibly chemical reorganization."

Turner (1930), also working on *Euplotes patella*, agrees with Griffin regarding the absence of granules in the solution plane and considers the substance in it to be in the physical state of a colloidal solution. The chromatin reticulum in the center of the nucleus he interprets as

in the continuous phase while the nuclear karyolymph is dispersed. After action in the reorganization bands this condition is reversed, the chromatin granules being in the dispersed phase and the karyolymph in the continuous.

These phenomena indicate that, at division, a peculiar type of catalysis occurs in the macronucleus whereby the chromatin undergoes some kind of reorganization which leaves it in the condition of a young organism ready to divide. They are, moreover, expressions of a general wave of reorganization which passes over the entire organism, resulting in a more or less complete new set of derived structures for both anterior and posterior daughter cells. The underlying cause of this periodic and cataclysmic phenomenon is unknown but the facts are unmistakable.

The first careful observations on the happenings to the cortical structures of the derived organization were made by Wallengren (1900, 1901, 1902). Prior to this, Stein (1859), Sterki (1878), and others had observed and maintained that all of the motile organs and oral parts are renewed at division in a number of ciliates (*Holosticha, Stylonychia, Gastrostyla, Uronychia,* etc.). Wallengren carefully described the process for each of the cirri of *Stylonychia* and other ciliates and, with minor variations, fully confirmed the earlier observations. The latest observer, Turner (1930), finds that not only are all cirri of *Euplotes patella* absorbed and renewed, but that the coördinating fibers from the anal cirri are also included in the loss and regeneration of the elements of the neuromotor system. Contrary to previous observers, he finds that in *Euplotes* the original cell mouth is not renewed during division but continues to function throughout the process. Whether or not it is renewed shortly after division is not yet satisfactorily determined.

The phenomena described above as occurring in the relatively few types of ciliates that are cited probably occur in the same or in modified form in all types of Infusoria. Metabolic activities during interdivisional periods result in a changed organization in which a general wave of catalysis sweeps over the organism and ends in division of the cell as a whole. One effect of this wave is a restoration of the old macronucleus to its original post-cystic condition; another effect is the withdrawal of the structures of the old euplasmatic organization back into the fundamental organization and their replacement by new derived structures. Here, as with de-differentiation described by Child, the reorganization processes result in an increased rate of metabolism.

Flagellates, Sarcodina, and Sporozoa are less spectacular than the ciliates in phenomena of reorganization by division. Nevertheless there are some striking cases of reduction, absorption, and re-formation of

derived structures. In flagellates division of the cell is longitudinal so that differential regeneration, characteristic of ciliates, is absent.

While there are many cases on record where the old flagellum is retained by one of the daughter cells at division and a new flagellum grows out from the divided blepharoplast in the other daughter cell, there are many cases on record of absorption of the old flagellum or flagella, and re-formation of flagella from centrioles or blepharoplasts. The latter usually arise by division of the endobasal body of the nucleus as in *Spongomonas, Trichomonas,* etc.

One of the best-supported cases in the latter category is the genus *Lophomonas,* an intestinal parasite of the cockroach. Here the spherical (*L. blattarum*) or elongate (*L. striata*) cell has a prominent brush of flagella at the anterior end. Each flagellum comes from a basal body and each basal body from a short rod-form blepharoplast. The group of blepharoplasts form a collar at the anterior end of the cell. Each blepharoplast gives rise posteriorly to a fibril (rhizoplast) and the group of fibrils form a basket or calyx in which the nucleus lies. Posterior to the nucleus the fibrils come together to form an axial strand which runs to the posterior end of the cell.

When ready for division, the nucleus emerges from its basket, centrioles derived by division of the endobasal body appear on the outside of the nuclear membrane, and a spindle is formed between them. The nuclear complex now migrates to the posterior end of the cell and a minute group of blepharoplasts arises from each centriole. The nucleus divides and the two daughter nuclei, each with its newly-formed group of blepharoplasts, migrate towards the anterior end of the old organism, one on each side of the division plane. The old aggregate of flagella, basal bodies, and blepharoplasts disappears by absorption in the protoplasm of one of the daughter cells, each of which has a newly-formed group of flagella, collar, calyx, and axial strand. In principle this is the same type of phenomenon as in ciliates and recalls the loss of the old mouth and oral basket of trichites, and the formation of two new mouths and baskets during the division of *Chilodon uncinatus* as described by MacDougall (1925).

In the majority of the Sarcodina and Sporozoa binary division is not the usual mode of reproduction. Where it does occur, as in Amœbidæ, noticeable derived structures are absent and reorganization, if it occurs, is confined to the cytoplasmic make-up of which we have little evidence. In Testacea there is a modified type of division, so-called budding division, during which water is absorbed by the protoplasm of the old cell. This is accompanied by lively cyclosis and protrusion of a protoplasmic bud from the shell mouth. This bud grows, assumes the shape of the

parent shell, and secretes its own chitinous membrane on which foreign particles (Arcellidæ) or plates manufactured by the parent protoplasm (Euglyphidæ) are glued. Apart from the withdrawal of old pseudopodia there is no visible evidence of reorganization. In Heliozoa at division, pseudopodia with their axial filaments are drawn in and new ones are formed by the daughter cells.

In the vast majority of Sarcodina (Radiolaria, Foraminifera, and Mycetozoa) and in most Sporozoa, binary division is replaced by multiple division. Nuclei divide repeatedly and a portion of the cytoplasm is ultimately parcelled out to each of the daughter nuclei. The minute cells thus formed leave the parent organism as swarm spores. Metabolic products, waste matters, and structures of the derived organization are abandoned and the shells and skeletons alone record the existence of the previously living cells. Each spore has only the fundamental organization together with a new potential of vitality; it is equivalent to the reorganized daughter cell of a divided ciliate.

Inadequacy of Reorganization by Division

It might seem that the method of restoring protoplasm to its fundamental organization by division would suffice to explain the indefinite longevity of Protozoa. This, however, is not the case. It is true that in animal flagellates this is the only method known, but it is not true of other groups. In ciliates the power to divide, if other methods of reorganization are prevented, gradually weakens; the inter-divisional periods are progressively lengthened; degeneration of the derived structures finally results. The micronucleus hypertrophies (Calkins, 1904) or disappears (Maupas, 1888) and the motile organs are lost (Maupas, 1888). In *Uroleptus mobilis* the chromatin of the macronucleus ultimately disappears and only a few large X granules remain. The protoplasm dies from what appears to be old age.

The effects of continued division in ciliates, with the exclusion of other methods of reorganization, are clearly shown by the method of isolation cultures. A single individual, preferably an ex-conjugant, is isolated in a suitable medium in a culture dish. The next day such an individual, for example a *Uroleptus mobilis,* is represented by four or eight daughter individuals, the number depending upon two or three divisions of the original one isolated. Five of these individuals are separately isolated in culture dishes, thus starting five lines all of which represent the protoplasm of the original ex-conjugant. A single individual is isolated daily from each line and the daily record of divisions is kept for all lines. The total number of divisions in all lines is averaged for 10-day periods. The division rate is taken as a measure of

vitality and the history of vitality is obtained by plotting the consecutive 10-day averages on a graph where the ordinates represent the number of divisions, the abscissas the consecutive 10-day periods. Such a graph for *Uroleptus mobilis* is shown in Fig. 5. There is a high initial vitality which gradually wanes through some 360 ± divisions during 300 ± days. In these isolation experiments the individuals do not encyst nor do they conjugate, hence division is the only means of reorganization. That division is not effective in checking the waning vitality is shown by the fact that, without exception, all such cultures die.

There is no doubt that the macronucleus is the most important of all derived structures of the ciliate organization. With each division

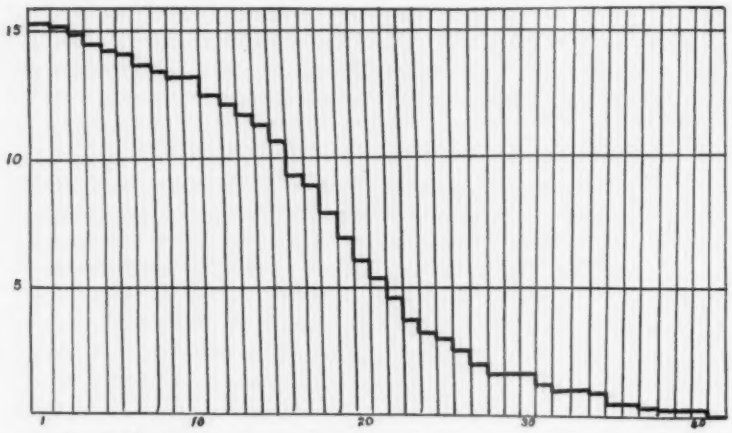

FIG. 5. Vitality graph of *Uroleptus mobilis*. A composite graph of twenty-three series averaged for successive 10-day periods.

of the cell, as shown above, it undergoes a process of reorganization whereby the chromatin is restored to a virile condition. With *Uroleptus mobilis*, in isolation cultures, this is repeated upwards of 350 times until reorganization, if it occurs, is ineffective and the protoplasm dies. It is probable that the macronucleus, like all derived euplasmatic structures, has a limited potential of activity. Other derived structures are periodically renewed from the fundamental organization. So too, under favorable conditions, is the macronucleus, the renewal taking place during the processes of endomixis and of conjugation.

In isolation cultures, if the extra individuals which remain over after the daily isolation is made on any day are placed in a larger container with abundant food, a so-called "encystment test" or "conjugation

test" is started. Here the individuals multiply by division until there may be thousands in the container. In the early stages of the life cycle of *Uroleptus mobilis* all such individuals ultimately die of starvation, but after approximately a month or six weeks after conjugation such tests result in an increasing number of encystments. A type of catalysis which is not seen in the division phenomena is now manifested. The macronucleus is broken down by fragmentation and the fragments are absorbed in the protoplasm while a new macronucleus is formed from a product of micronuclear division. Other structures of the derived or-

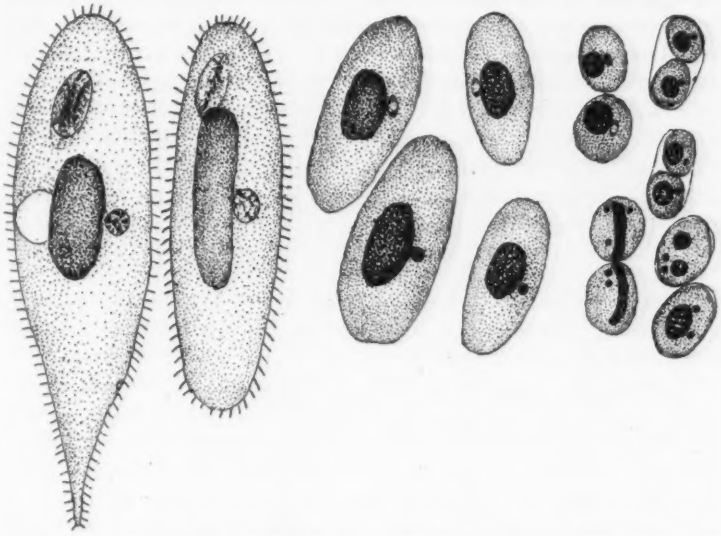

FIG. 6. *Glaucoma* (*Dallasia*) *frontata*. History of a normal vegetative individual with successive divisions resulting in copulating gametes and encysted zygotes (see Calkins and Bowling, 1929).

ganization are absorbed, waste matters and water are voided to the outside, and a cyst membrane is formed within which the organism may remain in a desiccated condition for months. When it is brought out of the cyst and established in isolation culture, it has an optimum vitality and passes through a complete life cycle exactly like that of an ex-conjugant. This phenomenon of endomixis, except for encystment, is the same as endomixis in *Paramecium aurelia* originally described by Woodruff and Erdmann (1914). Endomixis thus brings about a more far-reaching and a more complete reorganization than does division, and the important derived structure, the macronucleus, is renewed.

An interesting and illuminating variation, which I regard as equivalent to endomixis, is shown by the ciliate *Glaucoma* (*Dallasia*) *frontata*. At one stage in its life history, a stage corresponding to the period of encystment in *Uroleptus*, *Glaucoma frontata* loses its normal form and its normal movement and becomes elongate and cylindrical, darting about in the medium with unusual speed, (Fig. 6). Soon its mouth and complicated oral membranes are absorbed and it divides into two ellipsoidal individuals. These divide into four, the four into eight, and the eight into sixteen individuals without any intervening growth. These sixteen have no resemblance to the earlier parent form but each has a macronucleus and a micronucleus which has undergone reduction divisions and is haploid as to chromosomes. The sixteen are associated in pairs which become surrounded by a cyst membrane within which they fuse (Calkins and Bowling, 1929). This series of divisions is comparable with the process of endomixis in *Paramecium* with the exception that in the latter the micronuclei divide without division of the cell body. It further indicates that in ciliates generally, endomixis is a reminiscence of a gamete brood.

Reorganization by Conjugation

The longevity of a ciliate's protoplasm in the past is evidenced by the fact that it is before us today and has the possibility of indefinite longevity in the future. We know very little about the ultimate secrets of protoplasmic organization which underlie continued life, but we can analyze and control some of the conditions under which it keeps going. A proper environment and adequate food are essential factors. These lead to growth and to reproduction by division. We have seen above that the processes of division involve reorganization and the return of the protoplasm to a labile condition with a higher potential of metabolic activity. The periodic recurrence of these processes might well be adequate to ensure the longevity of the protoplasm, as seems to be the case with animal flagellates. It may well be the case, also, with some ciliates, but it must be remembered that the central organoid of metabolic activity—the macronucleus—is itself a derived structure and, presumably like other derived structures, is subject to absorption and replacement by a new macronucleus derived from the fundamental organization. This replacement does not occur at division, but, as shown above, it undergoes its own reorganization which we have likened to a nuclear purification. At endomixis, however, it follows the fate of other parts of the derived organization; a new one is formed from the fundamental organization and the result, again, is an increased vitality of the protoplasm. This phenomenon, recurring every thirty days, ensured the

longevity of *Paramecium aurelia* in Woodruff's hands for many years and through some thousands of generations by division. This phenomenon, indeed, as Woodruff states, may be adequate to ensure indefinitely continued life in the future, as division alone is adequate for flagellates.

In a conjugation test made with *Uroleptus* when the series is sixty or more days old the individuals multiply by division until there may be thousands in the container. After four or five days the bacterial food is diminished or even exhausted, and, if the race is "mature" an epidemic of conjugation occurs. Two individuals fuse at the anterior ends and remain united for about twenty-four hours (Fig. 7). They

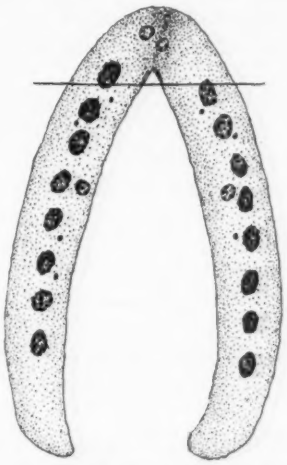

FIG. 7. *Uroleptus mobilis*, conjugating. The wandering pronuclei are passing each other at the anterior end. The line indicates the plane of cutting in merotomy experiments (see Calkins, 1921).

then separate and each of the two begins a process of reorganization which lasts for four or five days. If such an ex-conjugant is isolated, it will give rise to a new series and its progeny will pass through all the stages of vitality that the parent series passed through. At the outset its vitality will be the same as the original vitality of the parent series, but it will be greater than that of the parent series at the time of conjugation. It passes through the same history of waning vitality and its protoplasm finally dies, although this may be months after death of the parent protoplasm. This latter fact led Woodruff and Spencer (1924) to conclude that the effect of conjugation is to impart a certain "sur-

vival value" to each of the ex-conjugants. Vitality, however, means more than survival or life continued beyond that of the parent protoplasm. There are at least two factors involved—intensity of vitality and endurance (Calkins, 1920). A series living 100 days and dividing 150 times has an intensity of vitality represented by an average of 1.5 divisions per day. Another series living 300 days and dividing 450 times has the same average intensity of 1.5 divisions per day, but no one would say that the two series have the same vitality. Endurance (survival value) and intensity must be taken together in order to measure vitality, and both are increased as a result of conjugation.

These experiments with *Uroleptus,* continued for more than ten years and with 146 different series, invariably gave the same result. Each series lived for a period of from three months to a year or more and all were descendants of a single bit of protoplasm making up the body of one *Uroleptus* cell.

There is little reason to doubt that some change in the protoplasmic make-up occurs with continued metabolism through many successive generations by division. In isolation cultures it is manifested by an increasing time interval between divisions, i.e., a decreasing division rate, and by a change in the physical properties of the protoplasm whereby fusion of cells, total or partial, is possible. A type of catalysis not manifested earlier in the life history is set up. The cortex is liquified, usually in the region of the peristome, and two individuals fuse in conjugation at this region. In some cases the entire cortex is similarly liquified and individuals will fuse at any point on the periphery. I have seen no less than nine *Paramecium caudatum* thus fused into one amorphous mass.

The stimulus of normal fusion results in activities not manifested before. The micronuclei divide as they do in endomixis and their divisions involve reduction in number of chromosomes and a mutual interchange of pronuclei which meet and fuse. This activity of the micronuclei is generally interpreted as a reminiscence of an ancestral gamete brood which is realized in *Glaucoma frontata* and in gregarines. In the latter each individual of a syzygy forms a brood of gametes which copulate with similar gametes from the other individual of the pair.

Coming at the end of a vegetative period during which single or multiple divisions may have occurred, such gamete broods indicate a change in organization which, if copulation does not occur, results in death of the gametes. In other words, gametes are so specialized that they require a complemental combination to ensure normal metabolism. In some, differentiation has been in the direction of greater constructive activity resulting in a food-stored macrogamete or egg cell; in others,

in the direction of more catabolic activity resulting in a brood of microgametes or sperm cells as in Coccidiomorpha.

The happenings at conjugation in ciliates need only be recapitulated, for the phenomena are so well known that detailed description is unnecessary. Shortly after fusion of the anterior ends the micronuclei in both individuals begin a series of maturation divisions. The third division gives rise to the gametic nuclei. One of a pair of these remains in situ, the other migrates through the protoplasmic bridge, unites and forms an amphinucleus with the quiescent gametic nucleus of the other organism. A mutual fertilization results, the two wandering pronuclei in *Uroleptus* passing each other at one stage at the apex of the united pair. After union of the wandering and the quiescent pronuclei the two individuals separate, the amphinucleus divides twice, and one of the products forms a new macronucleus which, after four or five days, is ready to divide. Another product forms a new micronucleus. In the meantime the old macronucleus begins to degenerate and it ultimately fades away in the cytoplasm. The other structures of the derived organization of the old organism are absorbed and replaced by new ones. With this new make-up the young organism starts a new life cycle with an optimum of vitality.

Through these phenomena of conjugation an old protoplasm with low vitality is made over out of its own contained substances into a new protoplasm with high vitality. What explanation of this remarkable change in vitality can be given? The only apparent differences between the happenings at division and those at conjugation are: (1) fertilization, and (2) loss and replacement of the old macronucleus. Renewed vitality after conjugation may be due to one or the other or to both of these phenomena. Conjugation differs from endomixis only in catalysis of the cortex and in amphimixis. Fertilization, with its sequelæ of hereditary possibilities, is assumed to be the raison d'être for all sorts of subsequent peculiarities, but for the phenomenon of increased vitality under consideration a very simple experiment with *Uroleptus mobilis* shows that fertilization, with amphimixis, has nothing to do with the problem. A conjugating pair is cut across the apex with a scalpel. One individual of the two thus separated is fixed and stained to show the stage in conjugation when cut; the other individual is cultivated in an isolation culture dish. The experiment is particularly significant when the two wandering nuclei are cut off with the apical piece (Fig. 7). The cultivated individual goes through exactly the same processes as though conjugation had been completed in a normal manner; the old derived structures, including the macronucleus, are absorbed and replaced; the same characteristic renewal of vitality, and the same normal

life cycle occur, but amphimixis had been prevented—conjugation had been turned into endomixis (Calkins, 1921).

The second alternative, viz., the loss and replacement of the old macronucleus together with that of the old derived structures generally, remains as a possible explanation. This phenomenon is common to endomixis, conjugation, and conjugation merotomy, and in all cases a renewed vitality results. It does not happen in division although, as shown above, characteristic changes occur in the macronucleus. With its disintegration and absorption in the cytoplasm a new supply of nucleo-proteins is distributed in the cell and this may be a potent factor in the new vitality. Added to this is the fact that an entirely new and powerful organ of the cell—the macronucleus—has been supplied from the fundamental organization. The majority of the derived structures of the organism have a relatively short life, being absorbed and renewed at division (cirri and other motile organs); others, such as membranelles and oral structures, apparently have a longer life (especially anterior cells at division; posterior daughter cells always have newly-formed oral structures where the mouth is anterior). Of all derived structures the macronucleus has the longest life; it divides at cell division and may remain functional through entire life cycles including many hundreds of generations by division. It is the chief metabolic agent of the cell, while, in amicronucleate races, it is the only nucleus present. Yet it apparently lacks the possibility of continued life which the micronucleus possesses. It is essentially somatic in character and, like the soma of Metazoa, it ultimately wears out; if not replaced, the rest of the cell, including the micronucleus, dependent upon it, dies with it. It is quite possible, indeed I regard it as probable, that the gradual deterioration of this important organ of the ciliate cell is the underlying cause of waning vitality and ultimate death of protoplasm in isolation cultures.

Summary and Conclusions

The factors controlling longevity in protozoan protoplasm may be summarized and enumerated as follows:

1. A suitable environment with an adequate food supply.
2. The metabolic activities of the individual producing a constantly changing organization.
3. The catalytic processes of cell division whereby a changed organization is restored, in part, to the fundamental organization from which new derived structures are formed.
4. The catalytic processes of endomixis whereby a changed organization is more completely restored to the fundamental organization from which new derived structures, including the macronucleus, are formed.

5. The catalytic processes of conjugation, affecting the entire organization but beginning with the cortex, whereby a changed organization is fully restored to the fundamental organization from which a complete new set of derived structures, including the macronucleus, is formed. Here, also, is the opportunity for a change, through amphimixis, in the fundamental organization, and with it the possibility of variations.

6. Encystment, associated in most cases with endomixis, whereby the fundamental organization, in a dried state, may withstand unfavorable environmental conditions for long periods.

The line of thought developed above brings us back rather closely to Weismann's interpretation of Protozoa as having within themselves all that is necessary for an indefinitely continued existence. He was undoubtedly right, but when we consider the complexity of the processes involved in this continuity we are forced to the conclusion that the generalization should not be limited to the Protozoa but is equally true, with different degrees of complexity, in Metazoa and, indeed, in all other living things. His further assertion that Protozoa are equivalent to the germ plasm in Metazoa is true only in part. Had he limited it to the fundamental organization as we find it in the encysted organism, or periodically during the life history, we would find no objection to the generalization. Such an organism, like the egg cell, undergoes development, and new structures, often highly specialized and complex, are formed. Such structures constitute the soma which is analogous to the soma of Metazoa. Unlike the latter, however, the protozoan soma is reversible in greater or less degree and may be periodically renewed. When the conditions necessary for such reversion are inaccessible, as in isolation cultures with ciliates, the protoplasm dies a natural death, as in the case of the Metazoa, through waning of the potential vitality of the derived organization.

We cannot agree with Maupas' conclusion expressed in the title of his great work, "*Sur le rejeunissement karyogamique chez les ciliées*" (1889), for the old macronucleus is not rejuvenated in most cases at least in conjugation but is replaced by an entirely new one. It focusses attention, however, upon the micronucleus as the last stronghold of the fundamental organization in the struggle for continued existence.

Woodruff was right in the conclusion in his and Erdmann's paper of 1914, "A Normal Periodic Reorganization Process without Cell Fusion in Paramecium." It is probable that endomixis, with cell division, is adequate for continued existence of some ciliates, as division alone is adequate for continued life of animal flagellates. It is not, however, a

specific phenomenon of Protozoa, for its counterpart, parthenogenesis, is characteristic of many Metazoa.

Fertilization in Protozoa is an expression of an almost universal phenomenon of life. In all cases it involves the fusion of two minute bits of the protoplasmic fundamental organization. These may be in the form of isogametes or anisogametes, according to the type of specialization, or in the form of minute parts only of the fundamental organization, as in pronuclei of ciliates in conjugation, or pronuclei in Cnidosporidia. In Metazoa, and in Coccidiomorpha amongst the Protozoa, fertilization involves a stimulation to development which is inhibited through specialization of the gametes. Its significance in protoplasmic longevity is problematical. For the majority of Metazoa and for Coccidia, it is, indeed, obligatory, but for many Protozoa, e.g., animal flagellates and after merotomy of conjugants in ciliates, it is quite unnecessary for continued vitality. In general its effect seems to be the restoration of the fundamental organization to a balanced condition through the union of diversely specialized gametes, together with the formation of new combinations which may result in different types of derived organizations.

Little more can be said about the fundamental organization. In the thousands of species of Protozoa each one has its own specific type of organization—and no one knows what this specificity is. Through the ages each such specificity has been retained and maintained. Each bit of the fundamental organization goes through the same series of activities—response to stimuli, food ingestion and digestion, growth. Hundreds of activities go on unseen in its substance and the organization changes with every one such activity; structures of the derived organization are formed as a result of metabolic activities and all of them, except the alloplasmatic, are periodically reversed and restored to the fundamental organization. In appearance the derived organization changes from time to time either as enduring modifications (Jollos, 1913) or as mutations. In short, the history of the fundamental organization is a history of ceaseless change, but—*" plus ça change plus ça reste la même chose."*

BIBLIOGRAPHY

CALKINS, G. N., 1904. Studies on the Life History of Protozoa. IV. *Jour. Exper. Zool.*, **1**: 423.

CALKINS, G. N., 1911. Regeneration and Cell Division in Uronychia. *Jour. Exper. Zool.*, **10**: 95.

CALKINS, G. N., 1920. Uroleptus mobilis Engelm. III. A study in vitality. *Jour. Exper. Zool.*, **31**: 287.

CALKINS, G. N., 1921. Uroleptus mobilis Engelm. IV. Effect of cutting during conjugation. *Jour. Exper. Zool.*, **34**: 449.

CALKINS, G. N., 1930. Uroleptus halseyi Calkins. II. The origin and fate of the macronuclear chromatin. *Arch. Protist.*, **69**: 151.

CALKINS, G. N., AND R. BOWLING, 1929. Studies on Dallasia frontata Stokes. II. Cytology, gametogamy and conjugation. *Arch. Protist.*, **66**: 11.

GRIFFIN, L. E., 1910. Euplotes Worcesteri sp. nov. II. Division. *Phillipine Jour. Sci.*, **5**: 315.

JOLLOS, V., 1913. Experimentelle Untersuchungen an Infusorien. *Biol. Centralbl.*, **33**: 222.

KIDDER, G. W., 1933. Studies on Conchophthirius mytili de Morgan. I. Morphology and division. *Arch. Protist.*, **79**: 1.

MACDOUGALL, M. S., 1925. Cytological Observations on Gymnostomatous Ciliates, with a Description of the Maturation Phenomena in Diploid and Tetraploid Forms of Chilodon uncinatus. *Quart. Jour. Micros. Sci.*, **69**: 361.

MAUPAS, E., 1888. Sur la multiplication des Infusoires ciliés. *Arch. zool. expér. et gén.*, **6**: 165.

STEIN, F., 1859. Der Organismus der Infusionsthiere, Bd. 1.

STEIN, F., 1867. Der Organismus der Infusionsthiere, Bd. 2.

STERKI, V., 1878. Beiträge zur Morphologie der Oxytrichinen. *Zeitschr. wiss. Zool.*, **31**: 29.

SUMMERS, F. M., 1935. The Division and Reorganization of the Macronuclei of Aspidisca lynceus Muller, Diophrys appendiculata Stein, and Stylonychia pustulata Ehrenberg. *Arch. Protist.* In press.

TURNER, J. P., 1930. Division and Conjugation in Euplotes patella Ehrenberg with Special Reference to the Nuclear Phenomena. *Univ. Calif. Publ. Zool.*, **33**: 193.

WALLENGREN, H., 1900. Zur Kenntnis der vergleichenden Morphologie der hypotrischen Infusorien. *Bihang. till. Kongl. Svenska Vet.-Akad. Handl.*, **26**: 1.

WOODRUFF, L. L., AND R. ERDMANN, 1914. A Normal Periodic Reorganization Process without Cell Fusion in Paramæcium. *Jour. Exper. Zool.*, **17**: 425.

WOODRUFF, L. L., AND H. SPENCER, 1924. Studies on Spathidium spathula. II. The significance of conjugation. *Jour. Exper. Zool.*, **39**: 133.

YOCOM, H. B., 1918. The Neuromotor Apparatus of Euplotes patella. *Univ. Calif. Publ. Zool.*, **18**: 337.

FURTHER OBSERVATIONS ON THE DIURNAL MIGRATION OF COPEPODS IN THE GULF OF MAINE

GEORGE L. CLARKE

(From the Woods Hole Oceanographic Institution[1] and the Laboratory of General Physiology, Harvard University)

The following observations were undertaken to obtain further information on the diurnal migration of plankton in relation to environmental conditions. It was felt that the influence of temperature should be more carefully tested, particularly at the thermocline. The possibility that diurnal migration might be caused or controlled by reactions to food organisms required investigation. More precise and more extensive observations were desired on the effect of the changes in submarine irradiation with depth and with time of day. The observations were made at two stations in the Gulf of Maine, the first (Station 1722) occupied for 54 hours on July 14–16, 1933, in the deep central part of the Gulf but farther off shore than the stations of the previous year (Clarke, 1933), and the second (Station 1723) occupied for 24 hours on July 17–18, 1933, on the eastern edge of Georges Bank. By comparing the behavior of the copepods at these different localities, it was believed that the importance of differences both in environmental factors and in physiological condition of the animals would be revealed.

METHODS

The same methods were used for studying the migration as before with the following modifications. The nets were provided with new brass buckets in which the monel metal netting was coarser (18 strands/cm.), thus allowing faster drainage and minimizing clogging. The strap (15, Fig. 1*A*, Clarke, 1933) used to hold the tail of each net at the edge of its frame preliminary to lowering was replaced by a light brass chain through an appropriate link in which the pin (14) was inserted. The pursing rope (*P*) and the cord (18) were provided with spring clips for fastening them to the upper clamp (2) and the handline (19) respectively, thus saving the time formerly consumed in tying knots. To aid in keeping the ship in the same body of water throughout the whole period of observation, a buoy was constructed consisting of a keg with a pole thrust through it at right angles to its long axis. Since our intention was to study the plankton in the upper 50 meters

[1] Contribution No. 48.

of water particularly, a sea anchor was attached below the keg at a depth of 25 meters. The buoy therefore tended to stay with the body of water at that average depth, and since the keg floated three-quarters submerged, it had very little windage. The buoy was equipped with a flag during the day and with two hurricane lanterns at night and could be seen easily for several miles. The depth of the hauls is indicated in the tables. At the deep station the copepods migrated to such a depth that three series of hauls were sometimes necessary. The qualitative and quantitative analysis of the catch and the length measurements were very kindly carried out for me by Mr. Charles Wheeler assisted by Mr. David Lillie.

General Vertical Distribution

The catches were composed almost entirely of *Calanus finmarchicus* (Gunner). A small number of *Metridia lucens* (Boeck)—mostly adult females—was taken also in some cases (Tables I and II). At the deep station *Calanus* copepodid stage V was the most numerous group present. This group, together with copepodid stage IV and the adults of both sexes, were represented at some time of the day at every depth down to 174 meters. Very few individuals in copepodid stage III occurred at this locality. *Metridia* was generally confined to the deeper levels, although at night the range of the adult females and the immature did extend upwards to 6 meters. Since the water was 260 meters deep at this station, there was a considerable depth below the lowest haul which was not sampled at all. At the Bank station the adult females and copepodid stage V of *Calanus* were the most numerous group, but the adult males and stages IV and III were also well represented. All the *Calanus* groups were found at all levels. *Metridia* occurred chiefly at the deeper levels. Since bottom was at 65 to 75 meters at this station, the whole depth of water was sampled in this case.

To aid in distinguishing seasonal broods the cephalothorax length of copepods in the various sex and age groups were measured from representative hauls made at different depths and at different hours during the two stations (Table III). From Table IV it is seen that the ratio of the total length to the cephalothorax length of *Calanus* is extraordinarily constant. This means that relative shape remains the same in spite of differences in size.

The cephalothorax length of *Calanus* adult male changes very little with depth or with time of day. In the case of the adult females considerable constancy of length exists for all the hauls within each station, but at the deep station the averages range from 2.654 mm. to 2.722 mm.,

whereas at the Bank station the range is from 2.536 mm. to 2.674 mm. If this difference be regarded as significant, it may indicate that we are dealing with two different broods (*cf.* Russell, 1928, 1934; Marshall, 1933). None of the averages for the adult females is as low as that found for the 6-meter level previously, namely, 2.445 mm. (Clarke, 1933, Table VI). Furthermore, in the present case the range is not as great and there is not a distinct increase in length with depth. The average lengths of *Calanus* copepodid stage V show a little more variation at each station, but there is no consistent change with depth and no clear-cut difference between day and night hauls. The range of lengths is about the same at the two stations, and the average size is slightly greater than that found the previous year. Average lengths for copepodid stage IV range from 1.853 mm. to 1.903 mm. at the deep station and from 1.743 mm. to 1.867 mm. at the Bank station. The animals taken this season were generally larger than those of the same stage taken previously. The lengths of 25 specimens of *Calanus* copepodid stage III (Haul 420) ranged from 1.275 to 1.488 mm. and the average, 1.403 mm., is greater than that found the year before. For *Metridia lucens* the range for 27 specimens of the adult females from Haul 342 is 1.828 to 2.253 mm. and the average, 2.014 mm., falls within the range reported previously. In general the length measurements of all the copepods vary much less than was the case the year before and there are no regular changes within each group.

Environmental Conditions

The intensity of light present at different depths and its changes during the course of the day were measured at both the stations (Clarke and Oster, 1934). For the sections of the spectrum considered, red light had very nearly the same intensity as blue at the surface of the water but its intensity diminished very much more rapidly with depth. The intensity of red light was reduced to 1 per cent of its value at the surface at a depth of 11 to 12 meters whereas blue light did not suffer a corresponding reduction until a depth of 26 to 40 meters was reached. Since the copepods appear to have migrated downwards more than 100 meters at the deep station, it seems probable that in so far as their movement is a response to light, it is the blue component rather than the red which is chiefly concerned. Lines of equal intensity for blue light only have been represented in all the figures of diurnal migration.

At the deep station the temperature of the water was measured every 5 meters to a depth of 50 meters and the series was repeated 19 times

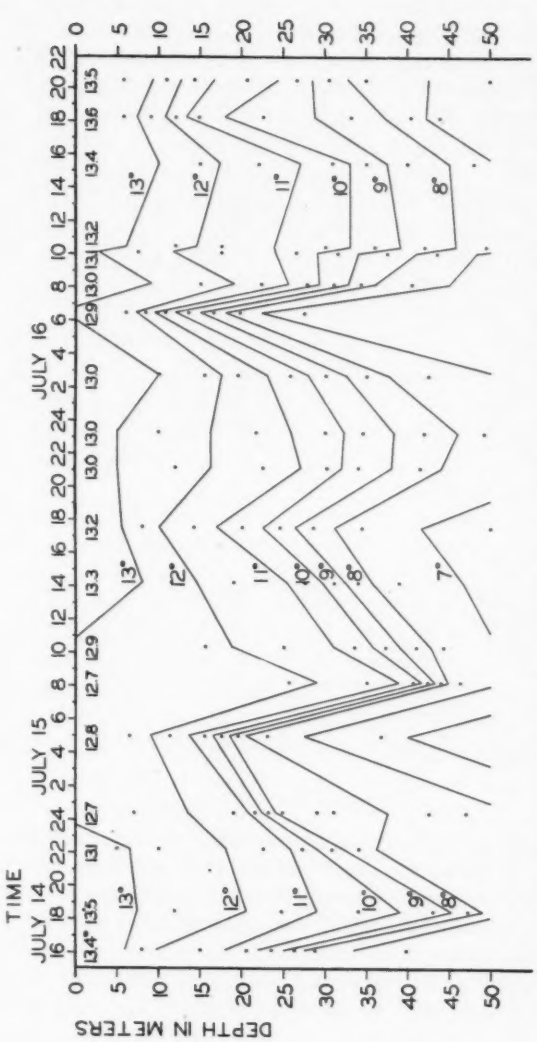

FIG. 1. Variation in temperature down to 50 meters at the deep station (Station 1722). Nineteen series of measurements were made at the hours indicated. The black dots indicate the half-degree points. The temperatures at the top are for the 1-meter level.

during the 54-hour period[2] (see Fig. 1). The isotherms show two sharp peaks: one at 5 o'clock, July 15, and the other at 6 o'clock, July 16. There are three less prominent peaks alternating with these.[3] The diurnal heating effect of the sun can be seen in the variation of the temperature at 1 meter. The difference between the highest and lowest temperatures at this depth was only 0.9° C. At a depth of 28 meters a change of 5° C. occurred in the 3 hours between 5 and 8 o'clock on July 15. Since it would be impossible for a large body of water to change its temperature so quickly, the effect must have been produced by a vertical movement of whole strata of water. This is substantiated by the fact that the thermocline is seen to have been periodically displaced. For example, during the 3 hours following 5 o'clock on July 15 it is moved downwards 25 meters. The temperatures below 50 meters at the deep station (Table I) were measured at three different times and found to vary on the average by about half a degree. For the one temperature observation taken at the Bank station the thermocline occurred between 15 and 20 meters. Data showing variation with depth of oxygen, phosphate, nitrate, and salinity for these stations are given by Braarud (1934, Tables I and II).

The vertical distribution of the phytoplankton at both stations has been reported by Braarud (1934). The phytoplankton was generally poor in both localities, but coccolithophorides, peridinians and ciliates were found above the thermocline and diatoms existed below it. At the deep station *Pontosphæra Huxleyi* was the dominant form and occurred in maximum numbers at 18 meters. Although this species was also the most numerous at the Bank station (maximum abundance at 15 meters), the dominant form in respect to bulk was the much larger *Guinardia flaccida* with a level of maximum abundance at 30 meters.

Diurnal Migration

The sex and age groups of *Calanus finmarchicus* all exhibited much the same type of diurnal migration at the deep station as may be seen from Figs. 2 and 3. The copepods of all groups were distributed chiefly below 80 meters at 1730 (Local sun time) on July 14. At 1930 there was an indication of an upward movement, but at 2130 maximum numbers occurred at the bottom of the series. Probably a rapid upward migration took place during the interval between these two series,

[2] In connection with meteorological investigations of Prof. C. G. Rossby and Mr. R. T. Montgomery.

[3] Similar fluctuations in temperature were observed by Prof. C. G. Rossby and Prof. A. C. Redfield on June 1, 1934 from the "Atlantis" while anchored at Station 2212 (not yet reported).

with the result that at 2130 the major part of the migrating population was present above 70 meters where no hauls were made. Support for this suggestion is furnished by the fact that copepods occurred in numbers in the upper strata at 20 o'clock on the following two evenings. Furthermore, since a large population existed near the surface at dawn on July 15, an upward migration must have taken place at some time during the night. The descent the following morning was very rapid, for at 0530 the maximum occurred at 66 meters or below and at 9 o'clock the entire population had sunk below 100 meters. At 1630 the upward movement had begun and proceeded rapidly until about 20 o'clock. Not much change in distribution took place during the remainder of the night, but in every case except that of the adult females, the population occurred at a slightly lower level before dawn the next morning (*cf.* Worthington, 1931; Nicholls, 1933). The migration on July 16 followed very closely the course run on the previous day. The indication of a concentration of adult males at the 6-meter level at 0430 on July 16 is probably due to a sampling error, the great majority of animals undoubtedly being below 54 meters at that time (*cf.* Table I). The general nature of the migration of *Calanus* at this station is similar to that observed by Nicholls (1933) for the adult females in the Clyde Sea-Area.

The slight differences in the behavior of the sex and age groups are chiefly matters of the distance through which the population moved. Thus, although all groups migrated to a depth of 120 meters or more during the day time, the adults descended farther than the immature on July 15. At night, the range of every group extended up to 6 meters but the level of maximum abundance was never higher than 42 meters for the males and never higher than 30 meters for copepodid stages V and IV, whereas for the females it occurred at 6 meters in the first series on July 16. Generally throughout the day the males were distributed deeper than the other groups.

A consistent though rough correlation between the vertical distribution of the copepods and the intensity of the submarine irradiation is apparent from the figures. Practically no individuals were taken from zones where the light intensity was 100 μw or greater. Early in the morning the center of the population coincides fairly closely with the 1.0 μw line, but in the next few hours the copepods descend more rapidly than the lines of equal intensity do, until at 9 o'clock maximum numbers occur at depths where the irradiation is less than 0.01 μw. It appears that insofar as the animals are reacting to light a higher intensity is required to initiate the migration than is required to cause it to continue. At 16 o'clock and thereafter the copepods were found in strata where

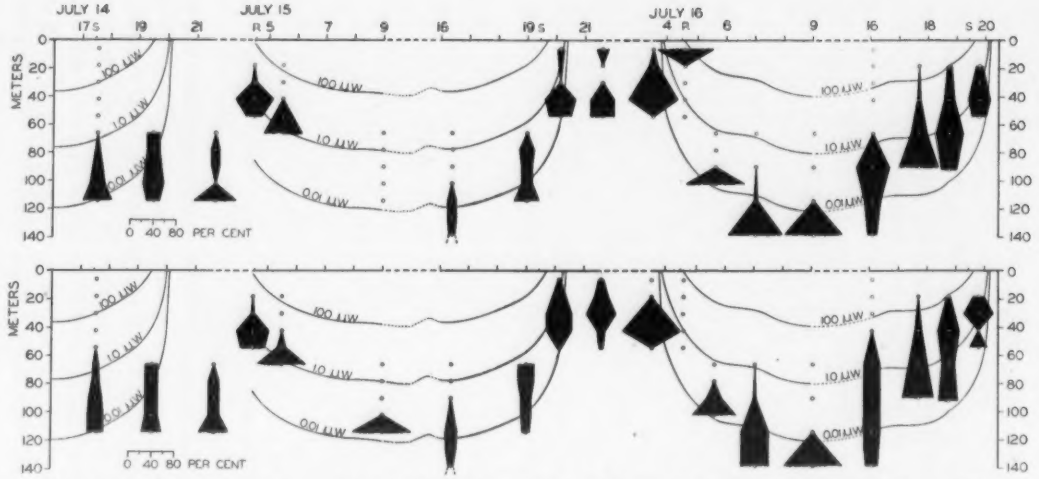

FIG. 2. Upper—*Calanus finmarchicus*, adult male. Lower—*Calanus finmarchicus*, copepodid Stage IV. The diurnal migration during the 54-hour period at the deep station (Station 1722). The black circles and white dots indicate the depths at which hauls were made. The changes in the intensity of the blue component of daylight are shown by the lines representing the depths at which 100 microwatts/cm.², 1.0 microwatts/cm.², etc. occurred. Where the lines are broken, a shortening of the time scale is indicated merely; observations were not discontinued during these periods. *S* = Sunset. *R* = Sunrise.

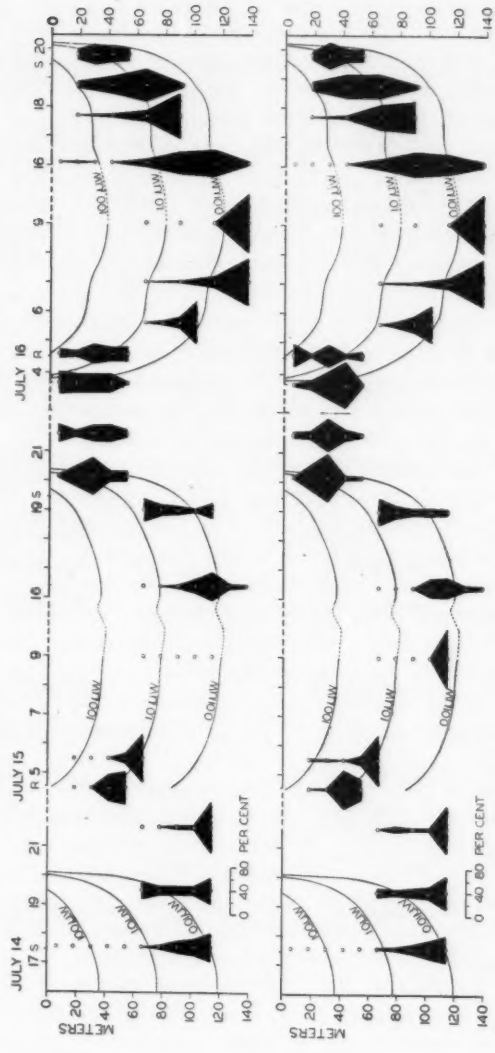

FIG. 3. Upper—*Calanus finmarchicus*, adult female. Lower—*Calanus finmarchicus*, copepodid stage V. The diurnal migration at the deep station (Station 1722).

the irradiation was more than 0.01 μw and in some cases even more than 1.0 μw. This tolerance of higher irradiation in the latter half of the day can be explained as the result of decreased sensitivity following light adaptation (*cf.* Worthington, 1931; Russell, 1931). *Calanus* copepodid stage III was almost entirely absent at the deep station.

Males, females, and immature of *Metridia lucens* occurred in the deep hauls and in the shallower hauls made at night. Toward the end of the afternoon on July 15 *Metridia* was encountered from 138 to 174 meters, and on July 16 from 90 to 138 meters. Females and immature reached the 6-meter level and males reached the 42-meter level at 2138 on July 15, but these strata were deserted early in the morning.

The observations made on the succeeding days at the Bank station revealed an entirely different vertical distribution and diurnal migration of *Calanus*, the major portion of the population being confined to the upper strata at all times (*cf.* Nicholls, 1933, p. 155; Russell, 1934). This station was about 100 miles south of the preceding and located in a physiographically distinct part of the Gulf, but, as described above, the environmental conditions were not profoundly different. The sex and age groups of *Calanus* all exhibited the same general behavior (Table II and Figs. 4 to 6). At 16 o'clock on July 17 maximum numbers occurred at depths of from 5 meters to 25 meters, and as night came on there was a slight movement upwards. At 3 o'clock the next morning most groups were rather evenly distributed in the upper 30 meters but an hour later there was indication of a concentration toward the surface again (*cf.* Russell, 1926). Soon after 6 o'clock the main downward migration began. The upper strata were progressively deserted during the remainder of the morning until at 14 o'clock maximum abundance occurred at a depth of 25 meters. By 1530 the copepods had started to move toward the surface again.

The adult males (Fig. 4, upper) did not migrate toward the surface as strongly as did the other groups of *Calanus*. The males were rather evenly distributed throughout the day and the maximum was usually to be found at 12 to 15 meters. The adult females (Fig. 4, lower) not only were much more abundant in the uppermost water layer but also were taken in even smaller numbers below 30 meters. This group exhibited little tendency toward uniform distribution during the night and did not leave the surface as early in the morning as did the males. The copepodid stage V population (Fig. 5, upper) was not highly concentrated, being well represented at all depths even down to 65 meters, but its vertical movement was slightly more extensive than that of the adults. Copepodid stage IV (Fig. 5, lower) migrated more strongly toward the

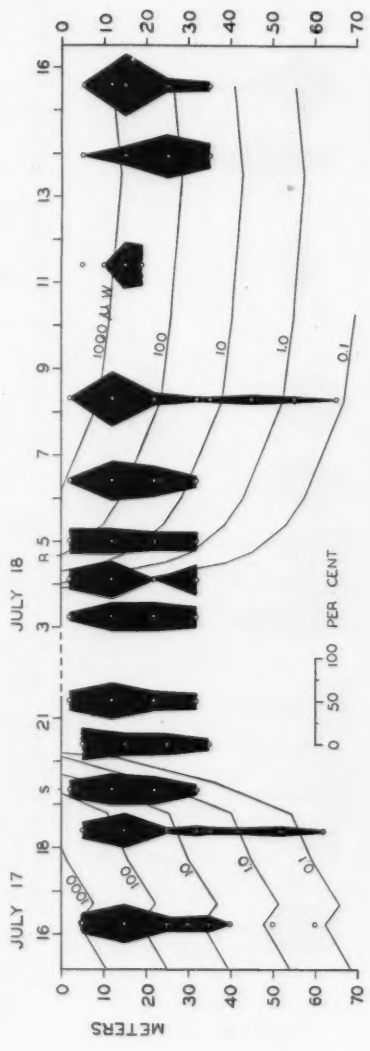

FIG. 4. Upper—*Calanus finmarchicus*, adult male. Lower—*Calanus finmarchicus*, adult female. The diurnal migration during the 24-hour period at the Bank station (Station 1723).

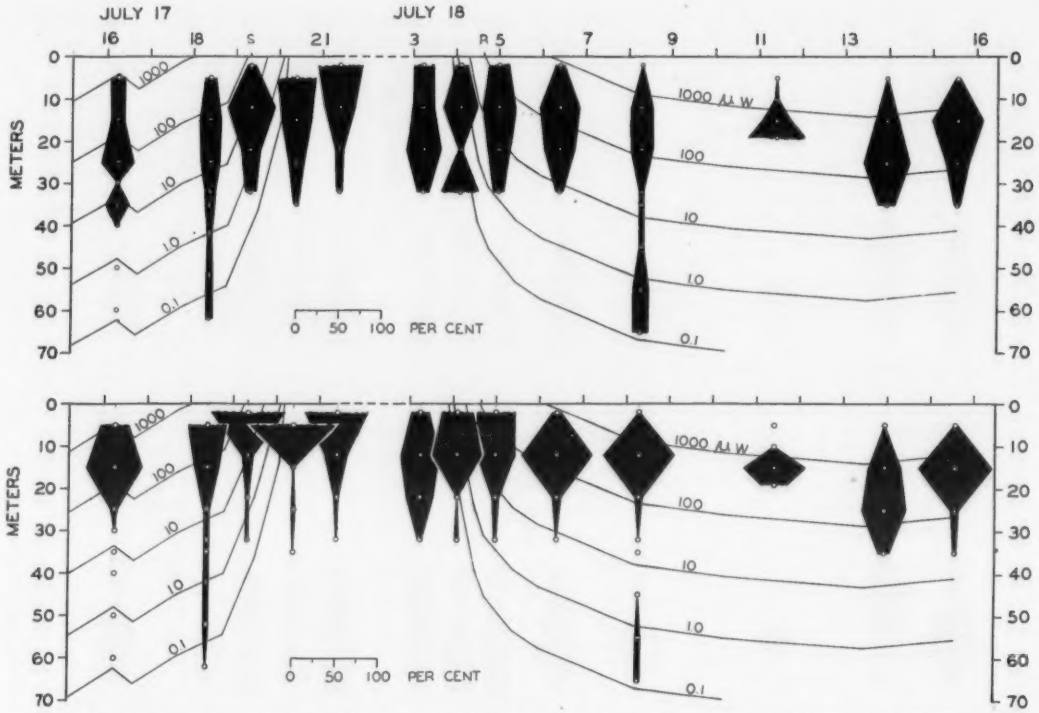

FIG. 5. Upper—*Calanus finmarchicus,* copepodid stage V. Lower—*Calanus finmarchicus,* copepodid stage IV. The diurnal migration at the Bank station (Station 1723).

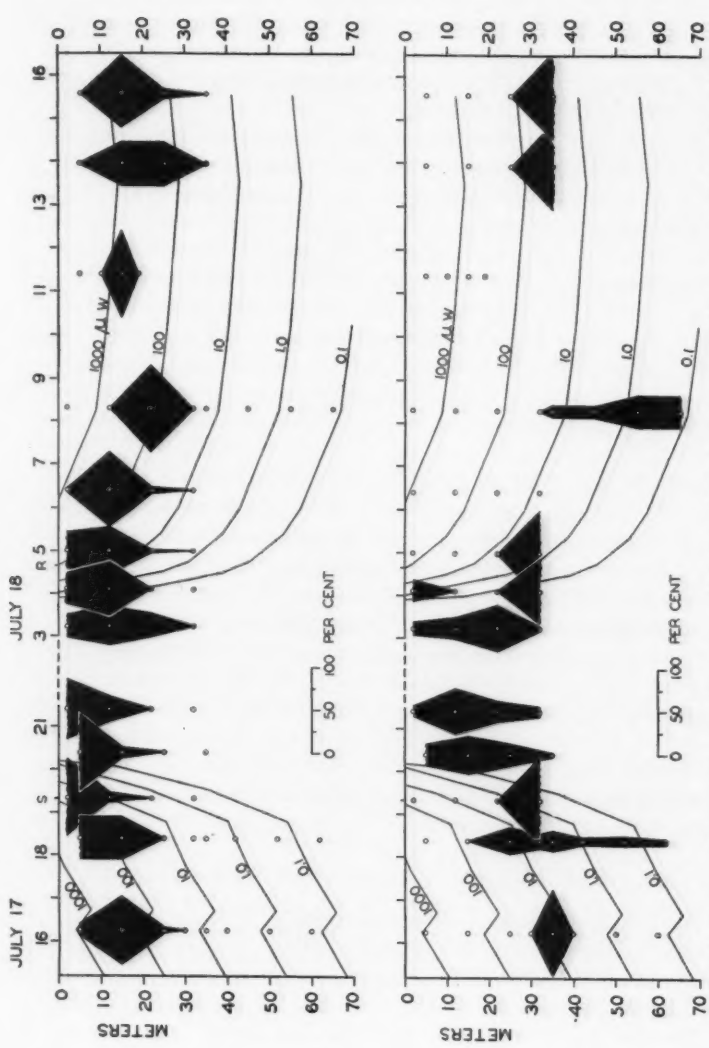

FIG. 6. Upper—*Calanus finmarchicus*, copepodid stage III. Lower—*Metridia lucens*, adult female. The diurnal migration at the Bank station (Station 1723).

surface at night than stage V and did not descend as far during the day. The tendency toward uniform distribution early in the morning is clearly marked. The distribution of copepodid stage III (Fig. 6, upper) was much the same as stage IV except that the level of maximum abundance never occurred below 22 meters and extremely few individuals were taken below 25 meters at any time.

While the *Calanus* groups were carrying out these very limited migrations at this station, the *Metridia* population showed a considerable vertical movement. During the first afternoon and evening the level of maximum abundance of the adult females (Fig. 6, lower) moved upwards from 35 meters to 12 meters. The animals did not continue migrating toward the surface during the night as previously (Clarke, 1933), for early the next morning the maximum occurred at 22 meters. A rapid downward movement followed until at 0830 the population was found chiefly below 35 meters. The adult males and immature of *Metridia*, which were taken in small numbers, appear to have migrated in much the same manner as the adult females, although not as extensively.

Since all the groups of *Calanus* remained in the upper water layers throughout the day at this station the animals must have been able to withstand strong irradiation. It can be seen from the figures that the copepods sometimes occurred in large numbers at levels where the light intensity was 1000 μw or more. However, the maximum abundance for all the groups of *Calanus* was usually found between the 1000 μw and the 100 μw lines. In most cases it is evident that the animals are inhabiting zones of higher light intensity in the afternoon than early in the morning, indicating that light-adaptation has taken place. The adult females of *Metridia* were confined to zones of lower irradiation. In the morning they appear to have avoided light intensities higher than 10 μw, but later in the day they were taken at levels where the irradiation was between 100 μw and 10 μw.

Discussion

The present observations illustrate the fact frequently pointed out by other investigators that the diurnal migration of plankton is a highly variable affair (Russell, 1932; Southern and Gardiner, 1932). The fact that the sex and age groups of *Calanus finmarchicus* all exhibited much the same type of behavior in the present case is in sharp contrast with the observations of Nicholls (1933) on this species made during his excellent investigations in the Clyde Sea-Area. At our deep station *Calanus* was found to be carrying out an extensive migration but at our

Bank station the same species exhibited a very slight diurnal movement. At the deep station the temperature was generally lower than at the Bank station, there being about 2° C. difference at the surface. Since *Calanus* is a cold-water species one would have expected a movement away from the surface in the warmer locality rather than in the colder. At the deep station the copepods carried out their migration in spite of the vertical oscillations of the water strata. The fact that the animals moved across the thermocline shows that in this case, at least, temperature is of minor importance in controlling vertical distribution (*cf.* Clarke, 1934*b*; Russell, 1927, p. 241). At the Bank station the copepods remained in large measure above the thermocline.

The salinity of the upper strata was slightly lower at the deep station than at the Bank station, but below 30 meters it was much the same at both localities. There was very little difference in other hydrographic conditions at the two stations (Braarud, 1934) and the differences in the phytoplankton do not appear to be significant although we do not know precisely which forms are important as food organisms (*cf.* Clarke, 1934*a*). The dominant form, *Pontosphæra,* was abundant above the thermocline in both cases, and the diatom population below the thermocline at the Bank station can be of no importance since the majority of the animals did not visit the lower strata. In view of this situation not only does it appear that the differences in the behavior at the two stations are not due to differences in the phytoplankton, but also it is difficult to understand how food could be an important factor in causing or controlling the diurnal migration which did occur at the deep station (*cf.* Worthington, 1931; Nicholls, 1933). In their upward movement the animals passed right through the zone of abundance of phytoplankton.

The year before in the Gulf of Maine at Stations 1285 and 1286 (Clarke, 1933) the adult females of *Calanus* exhibited no regular movement and copepodid stages V and IV migrated over a very limited range only. At Station 1287 the adult female population tended to rise and fall between 16 and 30 meters, but the copepodid stages showed very little tendency to migrate. In general in both cases the females and stages V and IV occurred in nearly equal numbers at all depths. The environmental conditions at the time when these observations were made were not strikingly dissimilar from those at Stations 1722 and 1723.

Metridia carried out distinct migrations at both Stations 1722 and 1723, although in the former case the vertical movement was more extensive. The species—particularly the adult females—is evidently more sensitive to light than *Calanus* since it was confined to zones of less intense irradiation. Furthermore *Metridia* arrived at the surface

later than *Calanus* and left the surface earlier at both stations. The migration of the adult females of *Metridia* was generally similar to that observed for this group the year before at Station 1287 but differed in certain details. At Stations 1722 and 1723 the copepods did not show a tendency to continue swimming toward the surface after complete darkness as they did on the previous occasion. Moreover, in the present case the downward migration was begun more than an hour before sunrise, and the animals descended during the day to much greater depths at Station 1722 than they did at Station 1287 where the water was correspondingly deep.

These differences in behavior must represent differences in the physiological condition of the organisms. In the case of the wide disparity in the distribution and migration of *Calanus* at the deep station and the Bank station, the conclusion seems inevitable that a fundamental difference in the reaction of the animals to light existed. It may be that we are dealing with two distinct broods, which differed in their physiological states (*cf.* Russell, 1928, 1934), although no clear indication of this appears from the length measurements. On the other hand, dissimilarities in the response to light might have been caused within comparable broods by the differences in the environmental conditions even though these are apparently slight. It is significant to note, however, that nothing in the environment at the Bank station prevented *Metridia* from carrying out a considerable migration. Although it is well known that differences in the environment often affect the reactions of different organisms in diverse ways, the present state of our knowledge of the specific action of external factors on copepods is insufficient to explain the variations in these observations on diurnal migration. However, we do know that phototropic reactions are sometimes under a very delicate control (Clarke, 1932).

We may conclude that in the control of the diurnal migration of copepods the environmental conditions are important from two points of view. First, they have a direct action in providing the stimuli by which the animals' swimming reactions may be evoked. Secondly, an indirect action is involved by which the factors of the environment influence the physiological condition of the organism. The environment is responsible for the physiological differences which may exist not only between seasonal broods but also between individuals of the same brood. Of the factors which directly affect the vertical movement of the copepods, light is the most important, but the migration may be indirectly influenced by the environment if conditions are such that the animals' reactions to light and to the other factors are changed.

Summary

1. Observations were made on the diurnal migration of copepods in relation to submarine irradiation and other external factors at a 54-hour and a 24-hour station in the Gulf of Maine using the same methods as previously. The zoöplankton consisted almost entirely of *Calanus finmarchicus* with a small number of *Metridia lucens*. The length of the copepods varied much less than was the case previously.

2. The variation with depth of the light intensity, the temperature, chemical constituents, and the phytoplankton was observed. During the first station the temperature was found to fluctuate widely.

3. At the station in the deep part of the Gulf all groups of *Calanus* and *Metridia* migrated to a depth of 120 meters or more during the daytime and moved upwards to levels of from 6 meters to 42 meters at night. At the station on Georges Bank *Calanus* was confined to the surface strata, undergoing only a very limited migration, but *Metridia* carried out an extensive migration.

4. The migration of the copepods was found to be more closely correlated with the changes in the submarine irradiation than with changes in the hydrographic conditions or in the phytoplankton. However, great variability in the behavior was observed. This appears to be due to differences in the physiological condition of the animals and represents the indirect action of the environment.

BIBLIOGRAPHY

Braarud, T., 1934. A Note on the Phytoplankton of the Gulf of Maine in the Summer of 1933. *Biol. Bull.*, **67**: 76.

Clarke, G. L., 1932. Quantitative Aspects of the Change of Phototropic Sign in Daphnia. *Jour. Exper. Biol.*, **9**: 180.

Clarke, G. L., 1933. Diurnal Migration of Plankton in the Gulf of Maine and its Correlation with Changes in Submarine Irradiation. *Biol. Bull.*, **65**: 402.

Clarke, G. L., 1934*a*. The Rôle of Copepods in the Economy of the Sea. Fifth Pacific Science Congress, June, 1933 (in press).

Clarke, G. L., 1934*b*. Factors Affecting the Vertical Distribution of Copepods. *Ecological Monographs*, **4**: 530.

Clarke, G. L., and R. H. Oster, 1934. The Penetration of the Blue and Red Components of Daylight into Atlantic Coastal Waters and its Relation to Phytoplankton Metabolism. *Biol. Bull.*, **67**: 59.

Marshall, S. M., 1933. On the Biology of Calanus finmarchicus. II. Seasonal variations in the size of Calanus finmarchicus in the Clyde Sea-Area. *Jour. Mar. Biol. Ass.*, **19**: 111.

Nicholls, A. G., 1933. On the Biology of Calanus finmarchicus. III. Vertical distribution and diurnal migration in the Clyde Sea-Area. *Jour. Mar. Biol. Ass.*, **19**: 139.

Russell, F. S., 1926. The Vertical Distribution of Marine Macroplankton. IV. The apparent importance of light intensity as a controlling factor in the behaviour of certain species in the Plymouth Area. *Jour. Mar. Biol. Ass.*, **14**: 415.

RUSSELL, F. S., 1927. The Vertical Distribution of Plankton in the Sea. *Biol. Rev. and Biol. Proc. Camb. Phil. Soc.*, **2**: 213.

RUSSELL, F. S., 1928. The Vertical Distribution of Marine Macroplankton. VII. Observations on the behaviour of Calanus finmarchicus. *Jour. Mar. Biol. Ass.*, **15**: 429.

RUSSELL, F. S., 1931. The Vertical Distribution of Marine Macroplankton. X. Notes on the behaviour of Sagitta in the Plymouth Area. *Jour. Mar. Biol. Ass.*, **17**: 391.

RUSSELL, F. S., 1932. Behaviour in Invertebrates. *Nature*, **129**: 449.

RUSSELL, F. S., 1934. The Vertical Distribution of Marine Macroplankton. XII. Some observations on the vertical distribution of Calanus finmarchicus in relation to light intensity. *Jour. Mar. Biol. Ass.*, **19**: 569.

SOUTHERN, R., AND A. C. GARDINER, 1932. II. The diurnal migrations of the Crustacea of the Plankton in Lough Derg. *Proc. Roy. Irish Acad.*, **40**: (Section B) 121.

WORTHINGTON, E. B., 1931. Vertical Movements of Fresh-water Macroplankton. *Int. Rev. Hydrobiol. und Hydrogr.*, **25**: 394.

TABLE I

Station 1722, Lat. 42° 50′ N., Long. 67° 05′ W.

Hauls *A*301–*A*320: July 14, 1933; sea: slight; sky: overcast; sunset: 1927.

Hauls *A*322–*A*360: July 15, 1933; sea: slight; sky: few clouds, fog; sunrise: 0433; sunset: 1926.

Hauls *A*361–*A*403: July 16, 1933; sea: slight becoming smooth; sky: overcast; sunrise: 0434; sunset: 1926.

Temperature: (See also Fig. 1) 54 meters, 6.50° C.; 72 meters, 6.25° C.; 90 meters, 5.57° C.; 113 meters, 5.74° C.; 136 meters, 6.01° C.; 158 meters, 6.53° C.; 181 meters, 6.90° C.; 202 meters, 7.06° C.; 227 meters, 7.02° C.

Numbers of copepods in hundreds; x = less than 50.

The data for each haul are given in the following order: haul number, depth (meters), local sun time; irradiation at surface ($\mu w/cm.^2$), irradiation at haul depth ($\mu w/cm.^2$), irradiation 30 minutes before (%): *Calanus finmarchicus* adult male, adult female, cop. V, cop. IV, cop. III; *Metridia lucens* adult male, adult female, immature.

*A*301, 6, 1706; 4000, 1900, 140: x, x, x, x, 0; 0, 0, 0
*A*302, 18, 1706; 4000, 390, 140: x, x, x, x, 0; 0, 0, 0
*A*303, 30, 1706; 4000, 110, 140: x, x, x, x, 0; 0, 0, 0
*A*304, 42, 1706; 4000, 34, 140: x, x, x, x, 0; 0, 0, 0
*A*305, 54, 1706; 4000, 8.4, 140: x, x, x, x, 0; 0, 0, 0
*A*306, 66, 1759; 1700, 1.0, 170: x, x, 3, 2, 0; 0, 0, 0
*A*307, 78, 1759; 1700, —, 170: 1, 5, 17, 3, 0; 0, 0, 0
*A*308, 90, 1759; 1700, —, 170: 2, 15, 46, 5, 0; 0, 0, 0
*A*309, 102, 1759; 1700, —, 170: 3, 22, 90, 6, 0; 0, 0, 0
*A*310, 114, 1759; 1700, —, 170: 6, 39, 141, 6, 0; 0, 0, 0
*A*311, 66, 1936; 44, —, 770: 3, 2, 3, 2, 0; 3, 15, 6
*A*312, 78, 1936; 44, —, 770: 3, 1, 2, 2, x; 3, 11, 10
*A*314, 102, 1936; 44, —, 770: 5, 1, 4, 2, 0; 7, 29, 3
*A*315, 114, 1936; 44, —, 770: 3, 3, 12, 3, x; 2, 10, 19
*A*316, 66, 2139; 0, 0, no light: x, x, x, x, 0; x, x, x
*A*317, 78, 2139; 0, 0, no light: 1, x, 6, 1, 0; 1, 1, 2
*A*318, 90, 2139; 0, 0, no light; 1, 1, 3, 1, 0; 1, 5, 2
*A*319, 102, 2139; 0, 0, no light: x, 1, 3, 1, 0; x, x, 1
*A*320, 114, 2139; 0, 0, no light: 5, 14, 50, 3, 0; 3, 8, 9
*A*322, 18, 0429; 140, 14, 6: x, x, x, 0, 0; 0, x, 0
*A*323, 30, 0429; 140, 3.8, 6: 1, 1, 7, 1, 0; 0, 0, 0
*A*324, 42, 0429; 140, 0.9, 6: 7, 66, 697, 24, 0; 0, 0, 0
*A*325, 54, 0429; 140, 0.3, 6: 3, 79, 366, 17, 0; 0, 0, 0
*A*326, 18, 0531; 1300, 130, 46: x, x, 1, x, 0; 0, 0, 0
*A*327, 30, 0531; 1300, 35, 46: x, x, 1, x, 0; 0, 0, 0
*A*328, 42, 0531; 1300, 11, 46: 0, 0, 0, 0, 0; 0, 0, 0
*A*329, 54, 0531; 1300, 2.7, 46: 1, 2, 24, 1, 0; 0, 0, 0
*A*330, 66, 0531; 1300, 0.7, 46: 2, 10, 137, 6, 0; 0, 0, 0
*A*331, 66, 0859; 7100, 4.0, 86: 0, 0, x, x, 0; 0, 0, 0
*A*332, 78, 0859; 7100, 1.1, 86: x, x, x, x, 0; 0, 0, 0
*A*333, 90, 0859; 7100, —, 86: 0, x, x, x, 0; 0, 0, 0
*A*334, 102, 0859; 7100, —, 86: 0, x, x, x, 0; 0, 0, 0
*A*335, 114, 0859; 7100, —, 86: x, x, 3, 1, 0; 0, 0, 0
*A*336, 66, 1558; 6200, 3.5, 130: 0, x, x, x, 0; 0, 0, 0
*A*337, 78, 1558; 6200, 0.9, 130: 0, x, x, x, 0; 0, 0, 0
*A*338, 90, 1558; 6200, 0.2, 130: x, 4, 24, x, 0; 0, 0, 0
*A*339, 102, 1558; 6200, —, 130: 0, 53, 263, 3, 0; 0, 0, 0
*A*340, 114, 1558; 6200, —, 130: 3, 121, 283, 7, 0; 0, 0, 0
*A*341, 126, 1652; 4700, —, 140: 3, 24, 56, 6, 0; 0, 0, 0
*A*342, 138, 1652; 4700, —, 140: 2, 10, 44, 3, 0; 1, 7, 10

TABLE I—*Continued*

*A*343, 150, 1652; 4700, —, 140: 3, 22, 47, 3, 0; 2, 7, 5
*A*344, 162, 1652; 4700, —, 140: 7, 10, 41, 6, 0; 4, 7, 4
*A*345, 174, 1652; 4700, —, 140: 3, 14, 93, 7, 0; 5, 3, 10
*A*346, 66, 1858; 810, 0.5, 150: x, 117, 1079, 23, 0; 0, 0, 0
*A*347, 78, 1858; 810, —, 150: 4, 57, 486, 23, 0; 0, 0, 4
*A*348, 90, 1858; 810, —, 150: 3, 41, 251, 14, 0; 0, 0, 0
*A*349, 102, 1858; 810, —, 150: 3, 17, 283, 14, 0; 0, 0, 3
*A*350, 114, 1858; 810, —, 150: 7, 83, 258, 10, 0; 3, 3, 17
*A*351, 6, 2009; 3.9, 1.8, 610: 2, 23, 76, 2, 0; 0, 0, 0
*A*353, 30, 2009; 3.9, 0.1, 610: x, 375, 2580, 42, 0; 0, 0, 0
*A*354, 42, 2009; 3.9, —, 610: 10, 104, 262, 41, 0; 0, 35, 10
*A*355, 54, 2009; 3.9, —, 610: 7, 104, 217, 10, 0; 3, 210, 35
*A*356, 6, 2138; 0, 0, no light: 4, 164, 312, 11, 0; 0, 8, 4
*A*357, 18, 2138; 0, 0, no light: 0, 61, 410, 27, 0; 0, 4, 0
*A*358, 30, 2138; 0, 0, no light: x, 150, 1855, 60, 0; 0, 0, 0
*A*359, 42, 2138; 0, 0, no light: 8, 163, 627, 15, 0; 4, 76, 53
*A*360, 54, 2138; 0, 0, no light: 8, 68, 213, 8, 0; 4, 125, 38
*A*361, 6, 0342; 0, 0, no light: x, 126, 14, 0, 0; 0, 2, 0
*A*362, 18, 0342; 0, 0, no light: 2, 111, 109, 0, 0; 0, 0, 0
*A*364, 42, 0342; 0, 0, no light: 11, 110, 567, 8, 0; 0, 4, 4
*A*365, 54, 0342; 0, 0, no light: x, 6, 32, x, 0; 0, 0, 0
*A*366, 6, 0434; 78, 39, 3: 1, 12, 25, 0, 0; 0, 0, 0
*A*367, 18, 0434; 78, 7.8, 3: x, 7, 3, x, 0; 0, 0, 0
*A*368, 30, 0434; 78, 1.2, 3: x, 28, 25, x, 0; 0, 0, 0
*A*369, 42, 0434; 78, 0.3, 3: x, 19, 5, 0, 0; 0, 0, 0
*A*370, 54, 0434; 78, —, 3: x, 21, 9, x, 0; 0, 0, 0
*A*372, 66, 0539; 1300, 0.7, 26: 0, x, x, x, 0; 0, 0, 0
*A*373, 78, 0539; 1300, —, 26: x, 2, 6, x, 0; 0, 0, 0
*A*374, 90, 0539; 1300, —, 26: x, 5, 17, 1, 0; 0, 0, 0
*A*375, 102, 0539; 1300, —, 26: 1, 19, 39, 3, 0; 0, 0, 0
*A*376, 66, 0702; 2800, 1.6, 61: 0, x, 1, x, 0; 0, 0, 0
*A*377, 90, 0702; 2800, 0.1, 61: x, 1, 4, 1, 0; 0, 0, 0
*A*378, 114, 0702; 2800, —, 61: 1, 17, 83, 11, 0; 0, 0, 0
*A*379, 138, 0702; 2800, —, 61: 15, 72, 349, 11, 0; 0, 4, 15
*A*380, 66, 0902; 7500, 4.3, 87: x, x, x, x, 0; 0, 0, 0
*A*381, 90, 0902; 7500, 0.3, 87: x, x, x, x, 0; 0, 0, 0
*A*382, 114, 0902; 7500, —, 87: 0, x, x, x, 0; x, 0, x
*A*383, 138, 0902; 7500, —, 87: 4, 4, 95, 23, 0; 6, 2, 21
*A*384, 6, 1547; 3900, 2000, 110: x, x, x, 0; 0, 0, 0
*A*385, 18, 1547; 3900, 390, 110: x, 2, 1, x, 0; 0, 0, 0
*A*386, 30, 1547; 3900, 59, 110: x, x, x, x, 0; 0, 0, 0
*A*387, 42, 1547; 3900, 15, 110: x, x, x, x, x; 0, 0, 0
*A*388, 66, 1628; 2800, 1.6, 130; 0, 48, 149, 4, 0; 0, 0, 0
*A*389, 90, 1628; 2800, 0.1, 130: 8, 144, 353, 4, 0; 0, 4, 8
*A*390, 114, 1628; 2800, —, 130: 4, 171, 281, 4, 0; 4, 4, 4
*A*391, 138, 1628; 2800, —, 130: 2, 14, 73, 3, 0; 2, 7, 12
*A*392, 18, 1743; 1200, 120, 200: x, 2, 5, 1, x; 0, 1, x
*A*393, 42, 1743; 1200, 4.6, 200: 3, 14, 47, 4, 0; 0, x, 1
*A*394, 66, 1743; 1200, 0.7, 200: 15, 80, 433, 11, 0; 0, 0, 0
*A*395, 90, 1743; 1200, —, 200: 36, 320, 515, 18, 0; 0, 0, 0
*A*396, 18, 1844; 910, 91, 130: 3, 57, 85, 2, 0; 0, 0, 0
*A*397, 42, 1844; 910, 3.5, 130: 7, 148, 248, 7, 0; 0, 0, 0
*A*398, 66, 1844; 910, 0.5, 130: 19, 281, 247, 4, 0; 0, 4, 11
*A*399, 92, 1844; 910, —, 130: 10, 30, 36, 6, 0; 3, 23, 17
*A*400, 18, 1948; 18, 1.8, 1300: 7, 221, 186, 7, 0; 0, 0, 0
*A*401, 30, 1948; 18, 0.3, 1300: 15, 426, 643, 19, 4; 0, 0, 0
*A*402, 42, 1948; 18, —, 1300: 24, 345, 322, x, 0; 0, x, 12
*A*403, 54, 1948; 18, —, 1300: 15, 274, 293, 11, 0; 0, 42, 19

TABLE II

Station 1723, Lat. 41° 20′ N., Long. 66° 40′ W.

Hauls *A*404–*A*431, July 17, 1933, Sea: smooth, heavy swell; sky: clear, fog; sunset: 1920.

Hauls *A*432–*A*467, July 18, 1933; sea: smooth, becoming slight, moderate swell; sky: few clouds, fog; sunrise: 0440; sunset: 1919.

Temperature: 1 meter, 15.45° C.; 5 meters, 15.47° C.; 10 meters, 15.39° C.; 15 meters, 13.92° C.; 20 meters, 10.34° C.; 25 meters, 8.67° C.; 30 meters, 8.47° C.; 40 meters, 8.44° C.; 50 meters, 8.21° C.; 60 meters, 8.07° C.; 70 meters, 7.98° C.

Numbers of copepods in hundreds; x = less than 50.

The data for each haul are given in the following order: Haul number, depth (meters), local suntime; irradiation at surface (μw/cm.^{2}), irradiation at haul depth (μw/cm.^{2}), irradiation 30 minutes before (%): *Calanus finmarchicus*, adult male, adult female, cop. V, cop. IV, cop. III; *Metridia lucens*, adult male, adult female, immature.

*A*404, 5, 1558; 3200, 1400, —: 10, 76, 31, 10, 2; 0, 0, 0
*A*405, 15, 1558; 3200, 190, —: 20, 55, 30, 20, 11; 0, 0, 0
*A*406, 25, 1558; 3200, 35, —: 8, 13, 65, 2, 1; 0, 0, 0
*A*407, 35, 1558; 3200, 7.0, —: 8, 4, 47, 0, 0; 4, 35, 5
*A*408, 30, 1632; 3700, 22, 87: x, x, x, x, 0; 0, x, 0
*A*409, 40, 1632; 3700, 4.8, 87: x, x, x, x, x; x, x, x
*A*410, 50, 1632; 3700, 1.0, 87: x, x, x, x, x; 0, 0, 0
*A*411, 60, 1632; 3700, 0.2, 87: x, x, x, x, 0; 0, 0, 0
*A*412, 5, 1759; 1400, 600, 120: 11, 34, 32, 8, 3; 0, 0, 0
*A*413, 15, 1759; 1400, 83, 120: 23, 42, 69, 5, 3; 0, 0, 0
*A*414, 25, 1759; 1400, 15, 120: 3, 4, 58, 1, 0; 0, 14, 1
*A*415, 35, 1759; 1400, 3.1, 120: 4, 2, 31, 1, 0; 0, 10, x
*A*416, 32, 1847; 650, 2.3, 170: 6, 4, 33, 1, 0; 0, 8, 0
*A*417, 42, 1847; 650, 0.7, 170: 4, 2, 14, 1, x; 8, 4, 1
*A*418, 52, 1847; 650, 0.1, 170: 6, 6, 19, 1, 0; 10, 5, x
*A*419, 62, 1847; 650, —, 170: 1, 2, 20, x, 0; 3, 3, 1
*A*420, 2, 1921; 99, 56, 530: 14, 86, 24, 77, 24; 0, 0, 0
*A*421, 12, 1921; 99, 13, 530: 25, 192, 143, 12, 3; 0, 0, 0
*A*422, 22, 1921; 99, 1.7, 530: 22, 61, 58, 1, 0; 0, 0, 0
*A*423, 32, 1921; 99, 0.3, 530: 8, 10, 33, 1, 0; 0, 12, 0
*A*424, 5, 2024; 0, 0, (5.2 μw): 14, 183, 121, 66, 14; 0, 7, 0
*A*425, 15, 2024; 0, 0, (5.2 μw): 10, 47, 99, 0, 2; 0, 12, 0
*A*426, 25, 2024; 0, 0, (5.2 μw): 9, 13, 47, 2, 0; 0, 7, 1
*A*427, 35, 2024; 0, 0, (5.2 μw): 5, 2, 15, x, 0; x, 1, 1
*A*428, 2, 2126; 0, 0, no light: 7, 87, 125, 34, 12; 2, 2, 0
*A*429, 12, 2126; 0, 0, no light: 14, 88, 85, 12, 5; 0, 9, 0
*A*430, 22, 2126; 0, 0, no light: 7, 10, 21, 1, x; 1, 4, 0
*A*431, 32, 2126; 0, 0, no light: 6, 3, 15, x, 0; x, 2, 0
*A*432, 2, 0315; 0, 0, no light: 3, 245, 52, 7, 3; 0, 3, 0
*A*433, 12, 0315; 0, 0, no light: 11, 23, 55, 15, 5; 0, 5, 0
*A*434, 22, 0315; 0, 0, no light: 11, 36, 91, 10, 3; 0, 10, 0
*A*435, 32, 0315; 0, 0, no light: 8, 6, 29, 1, 0; 1, 1, 0
*A*436, 2, 0406; 2.6, 1.5, no light: 3, 32, 7, 7, 5; 0, 1, 0
*A*437, 12, 0406; 2.6, 0.3, no light: 6, 27, 19, 11, 8; 0, 0, 0
*A*438, 22, 0406; 2.6, —, no light: x, 1, 1, x, 0; 0, x, 0
*A*439, 32, 0406; 2.6, —, no light: 5, 12, 20, 1, 0; 1, 3, 1
*A*440, 2, 0501; 380, 210, 16: 4, 75, 13, 31, 20; 0, 0, 0

TABLE II—*Continued*

*A*441, 12, 0501; 380, 49, 16: 4, 30, 22, 31, 26; 0, 0, 0
*A*442, 22, 0501; 380, 6.5, 16: 3, 10, 15, 5, 1; 0, 0, 0
*A*443, 32, 0501; 380, 1.3, 16: 3, 4, 7, 1, x; x, 1, x
*A*444, 2, 0626; 1600, 900, 81: 1, 26, 8, 3, 1; 0, 0, 0
*A*445, 12, 0626; 1600, 210, 81: 8, 40, 36, 25, 13; 0, 0, 0
*A*446, 22, 0626; 1600, 28, 81: 6, 20, 28, 3, 1; 0, 0, 0
*A*447, 32, 0626; 1600, 5.6, 81: 2, 2, 6, x, x; 0, 0, 0
*A*448, 2, 0802; 4900, 2800, 76: 0, 7, x, x, 0; 0, 0, 0
*A*449, 12, 0802; 4900, 640, 76: 13, 53, 30, 18, 6; 0, 0, 0
*A*450, 22, 0802; 4900, 85, 76: 3, 13, 31, 2, 0; 0, 0, 0
*A*451, 32, 0802; 4900, 17, 76: 1, 1, 5, 0, 0; 0, 0, 0
*A*452, 35, 0836; 6200, 14, 79: 1, x, 6, x, x; 1, 1, x
*A*453, 45, 0836; 6200, 4.0, 79: 2, 1, 5, x, 0; 1, 1, 1
*A*454, 55, 0836; 6200, 0.7, 79: 1, 1, 23, 1, x; 1, 3, 2
*A*455, 65, 0836; 6200, 0.1, 79: x, x, 22, 1, 0; 2, 3, 1
*A*456, 5, 1126; 11,500, 4900, 88: 0, x, x, x, 0; 0, 0, 0
*A*457, 10, 1126; 11,500, 2300, 88: 0, 1, 1, x, 0; 0, 0, 0
*A*458, 15, 1126; 11,500, 680, 88: 25, 60, 130, 15, 12; 0, 0, 0
*A*459, 19, 1126; 11,500, 320, 88: 22, 69, 259, 6, 0; 0, 0, 0
*A*460, 5, 1357; 10,800, 4600, 120: x, x, 0, 0, 0; 0, 0, 0
*A*461, 15, 1357; 10,800, 640, 120: 6, 30, 46, 6, 1; 0, 0, 0
*A*462, 25, 1357; 10,800, 120, 120: 14, 58, 84, 8, 1; 0, 0, 0
*A*463, 35, 1357; 10,800, 24, 120: 8, 11, 30, 2, 0; 0, 1, 0
*A*464, 5, 1535; 9000, 3800, 110: 1, 7, 12, 1, x; 0, 0, 0
*A*465, 15, 1535; 9000, 530, 110: 28, 91, 97, 28, 8; 0, 0, 0
*A*466, 25, 1535; 9000, 100, 110: 6, 28, 44, 3, 1; 0, 0, 0
*A*467, 35, 1535; 9000, 20, 110: 4, 3, 11, 1, x; 0, 1, x

TABLE III

Cephalothorax lengths of Calanus finmarchicus

	Station 1722									Station 1723							
	Day Hauls						Night Hauls			Day Hauls					Night Hauls		
	Haul 384 6 m.	Haul 386 30 m.	Haul 336 66 m.	Haul 339 102 m.	Haul 342 138 m.	Haul 345 174 m.	Haul 356 6 m.	Haul 358 30 m.	Haul 360 54 m.	Haul 404 5 m.	Haul 405 15 m.	Haul 408 30 m.	Haul 409 40 m.	Haul 411 60 m.	Haul 428 2 m.	Haul 429 12 m.	Haul 431 32 m.
Adult male																	
No measured.....	—	—	—	—	—	—	—	—	—	25	25	—	9	6	16	24	25
Average length mm.	—	—	—	—	—	—	—	—	—	2.506	2.504	—	2.508	2.416	2.553	2.455	2.514
Range.....	—	—	—	—	—	—	—	—	—	2.295–2.635	2.380–2.720	—	2.423–2.678	2.338–2.508	2.380–2.720	2.253–2.635	2.210–2.720
Adult female																	
No. measured.....	—	—	—	24	25	25	25	25	25	25	25	3	17	26	53	25	26
Average length mm.	—	—	—	2.722	2.710	2.693	2.654	2.715	2.669	2.581	2.606	2.621	2.588	2.536	2.674	2.640	2.621
Range.....	—	—	—	2.465–3.103	2.465–2.975	2.380–3.103	2.465–2.975	2.380–3.018	2.380–3.060	2.210–2.848	2.423–2.805	2.465–2.720	2.380–2.805	2.380–2.720	2.380–3.018	2.465–3.145	2.423–2.933

TABLE III—*Continued*

	Station 1722									Station 1723							
	Day Hauls						Night Hauls			Day Hauls					Night Hauls		
	Haul 384 6 m.	Haul 386 30 m.	Haul 336 66 m.	Haul 339 102 m.	Haul 342 138 m.	Haul 345 174 m.	Haul 356 6 m.	Haul 358 30 m.	Haul 360 54 m.	Haul 404 5 m.	Haul 405 15 m.	Haul 408 30 m.	Haul 409 40 m.	Haul 411 60 m.	Haul 428 2 m.	Haul 429 12 m.	Haul 431 32 m.
Copepodid V																	
No. measured.....	5	26	25	50	52	50	50	49	50	50	50	21	11	12	100	51	50
Average length mm.	2.431	2.240	2.220	2.424	2.460	2.399	2.422	2.388	2.436	2.417	2.458	2.374	2.241	2.271	2.434	2.329	2.350
Range.....	2.125–2.720	2.040–2.465	2.040–2.423	2.168–2.720	2.125–2.720	2.125–2.763	2.125–2.763	2.083–2.593	2.125–2.720	2.168–2.720	2.168–2.635	1.998–2.593	1.955–2.423	1.998–2.465	1.998–2.720	2.040–2.720	1.955–2.635
Copepodid IV																	
No. measured.....	—	—	—	—	25	25	25	12	12	25	25	1	1	1	50	26	2
Average length mm.	—	—	—	—	1.903	1.874	1.875	1.885	1.853	1.826	1.867	1.785	1.743	1.785	1.845	1.849	1.807
Range.....	—	—	—	—	1.785–2.040	1.700–2.338	1.658–2.040	1.828–2.083	1.785–1.955	1.615–1.955	1.743–2.083	—	—	—	1.700–1.998	1.700–2.083	1.785–1.828

TABLE IV

Comparison of Cephalothorax length and total length in Calanus finmarchicus

	Haul No.	No. measured	Average cephalothorax length	Average total length	Ratio
			mm.	*mm.*	
Adult male	431	25	2.508	3.230	0.777
Adult female	431	25	2.635	3.315	0.795
" "	358	25	2.720	3.400	0.800
Copepodid V	358	50	2.338	2.933	0.797
" IV	358	13	1.743	2.168	0.805

THE DIURNAL MIGRATION OF COPEPODS IN ST. GEORGES HARBOR, BERMUDA

GEORGE L. CLARKE

(From the Woods Hole Oceanographic Institution[1] and the Laboratory of General Physiology, Harvard University)

The variable nature of the diurnal migration of plankton has rendered difficult the task of discovering the importance of different environmental conditions in causing and controlling the phenomenon. Progress may be made, however, by comparing the behavior of the animals under different circumstances. In view of the data already obtained on the diurnal migration of copepods in the Gulf of Maine (Clarke,1933*b*, 1934), it appeared worthwhile to make similar observations in Bermuda waters, where different species of copepods and entirely different environmental conditions existed. The observations, made in St. Georges Harbor on January 23–24, 1934, must be regarded as approximations since only one (non-closing) net was used. The migration is of such magnitude, however, that there can be no doubt as to its general nature.

The net, kindly loaned to me by Dr. William Beebe, was of the international type. It was 50 cm. in diameter at the mouth and 3 m. long with very coarse netting at the upper end and silk bolting cloth at the middle (12 strands/cm.) and at the bottom (23 strands/cm.). A standard run of a little less than half a mile was laid out between two landmarks in St. Georges Harbor. The tows were made from the Laboratory launch run over this standard course at as nearly the same speed as possible, and always in the same direction, from west to east. Each plankton haul, therefore, was made through the same amount of water. The times required for the run ranged between 10 and 19 minutes, the variability being caused by changing wind conditions. In each set of observations, a haul was made at the surface first and this was followed as quickly as possible by a haul near the bottom. (In one case a third haul was made at a depth halfway to the bottom.) The depth of water was between 12.5 and 14 meters in this part of St. Georges harbor. For the deep haul the net was lowered until the weight, 2 meters below the net, struck the bottom. Then the launch was started and 2 meters more line was payed out. About 20 minutes elapsed between

[1] Contribution No. 54.

the surface and the deep hauls. The time stated in the table for each set is the midpoint between the times for the two hauls.

No attempt was made to study the hydrographic conditions in St. Georges Harbor in detail. The surface temperature was about 20° C. and since a tidal current flowed through the harbor, it is probable that the water was fairly well mixed and hence uniform. A surface haul and a deep haul were taken over the standard run with a diatom net (1 meter long, 30 cm. in diameter, netting 76 strands/cm.). The catches, very kindly analyzed for me by Dr. J. F. G. Wheeler, contained the following total numbers of organisms:

	Surface	Deep
Detritus	Most abundant	Most abundant but less than at surface
Chætoceras (chains)	19,000	27,000
Rhizosolenia	93,000	90,000
Flagellate A. (peridinian?)	79,000	131,000
14 other small forms	24,000	22,000

The analysis of the zoöplankton hauls—carried out by counting and identifying under the microscope either the whole catch or a sample of it—showed the presence of only two species of copepods: *Calanopia americana* (Dahl) and *Acartia spinata* (Esterly). These were divided into adult males and females. Immature specimens were encountered in some of the hauls but were too few to be treated quantitatively. *Calanopia* was found to be the dominant copepod in the bays and harbors of Bermuda at this time (*cf.* Esterly, 1911). It is true that this species was taken in the outside waters but in rapidly diminishing numbers with increasing distance from the mouths of the inland waterways. *Acartia*, on the other hand, was more numerous in the offshore waters and appeared to have been swept into the harbors by the tide.

In the diurnal migration of *Calanopia* and of *Acartia* (Table I) the males and females behaved in the same way. At mid-day the *Calanopia* population could not be found at any depth in the harbor. The animals were undoubtedly so close to the bottom at this time that they could not be caught by the plankton net. In fact, the copepods probably actually burrowed into the soft mud bottom, as is discussed below. At 1944 (local sun time) *Calanopia* was taken in large numbers in the surface hauls and in still larger numbers in the deep hauls. Early the next morning, while it was still dark, the relative distribution of the population was unchanged although all the catches were slightly smaller. Just before sunrise, the numbers of *Calanopia* at both levels were greatly reduced. Although no hauls were taken at noon on the second day, the water was undoubtedly entirely deserted just as before, and at 1707, a

quarter of an hour before sunset, the beginnings of the upward migration are apparent. Less than an hour later the numbers of *Calanopia* taken in the surface hauls had increased 10-fold and the numbers taken in the deep hauls had increased 100-fold.

TABLE I

Diurnal migration in St. Georges Harbor, Bermuda. Total numbers of copepods.

	Wind	*Sky*	*Sunrise*	*Sunset*
Jan. 23, 1934.....	SE. moderate	Many clouds	—	1724
Jan. 24, 1934.....	W. slight to strong	Clear	0700	1725

	Local Sun Time	Jan. 23, 1934		Jan. 24, 1934			
		1054	1944	0511	0656	1707	1801
Calanopia americana male	Surface.....	0	530	330	15	2	20
	Intermediate.....			2180			
	Deep.....	0	2750	2110	16	17	2340
Calanopia americana female	Surface.....	0	740	510	13	3	50
	Intermediate.....			2370			
	Deep.....	1	4380	2370	12	27	2620
Acartia spinata male	Surface.....	0	90	50	1	0	0
	Intermediate.....			60			
	Deep.....	30	30	90	120	1	4
Acartia spinata female	Surface.....	0	230	60	2	1	2
	Intermediate.....			130			
	Deep.....	40	50	140	180	2	4

The vertical migration of *Acartia* followed the general plan of the *Calanopia* migration but the differences between the day and night hauls are not as striking. At mid-day a considerable number of *Acartia* were taken by the deep net, indicating that this species did not descend to the bottom as completely as did *Calanopia*. At 1944 the upward migration had brought the majority of the animals to the surface. The first set of hauls on the following morning showed a downward movement and this had been greatly accelerated by sunrise. *Acartia* was scarce at 1707 at both levels and even at 1801 no considerable numbers had appeared. This disappearance of the *Acartia* population was coincident with the rising of a strong northwest wind which may have caused a removal of this species, which did not descend entirely to the bottom, through the harbor mouth to the east. On January 23 and on the preceding days, a very strong easterly wind had been blowing. It appears possible, then, that at the beginning of the investigation on diurnal migration there

may have been more of this oceanic species present in the harbor than normal, and that during the second day these copepods were being carried back to the outside waters.

Unfortunately it was not possible to make light intensity measurements, but it is clear that the diurnal migration of both *Calanopia* and *Acartia* shows a close correspondence with the rising and setting of the sun. The rapidity of the vertical movements of these copepods is in accord with the shortness of the duration of twilight in low latitudes. The clearness of the water in this part of the ocean (*cf.* Clarke, 1933*a*), coupled with the brilliance of the sun, must have resulted in an intensity of irradiation at all depths in St. Georges Harbor far greater than exists in the surface layers of the Gulf of Maine. It would not be surprising then to find that *Calanopia* had become adapted actually to burrow into the mud during the middle of the day. In fact, during culturing experiments in the laboratory it was found that when individuals of this species were introduced into small glass dishes of sea water with a layer of harbor mud on the bottom, they buried themselves almost immediately. Other species of calanoid copepods did not exhibit this behavior. The animals undoubtedly leave the mud at night for the purpose of feeding (*cf.* Worthington, 1931; Nicholls, 1933). This upward movement might appear superficially similar to the diurnal migrations of certain bottom-living animals (*cf.* Fage, 1933), but Dr. C. B. Wilson informs me that there is every indication that *Calanopia* is a truly pelagic form. As we have seen above, food organisms were present in the water of the harbor, although here as in other warm regions phytoplankton was generally scarce.

To sum up, the copepods *Calanopia americana* and *Acartia spinata* were found to exist in St. Georges Harbor in large numbers near the surface and in the deeper strata at night and to migrate to the bottom at sunrise, the former species apparently burrowing into the mud. As in the Gulf of Maine, the diurnal migration appears to be correlated primarily with changes in light intensity during the course of the day, although it may be partly under the control of other environmental factors as well. The fact that the copepods descend to the bottom is consistent with the great intensity of the submarine irradiation in the Bermuda region.

I am indebted to the Bermuda Biological Station for the use of the facilities of the laboratory and I wish to thank the Director, Dr. J. F. G. Wheeler, for his helpful coöperation during the course of this investigation.

BIBLIOGRAPHY

CLARKE, G. L., 1933*a*. Observations on the Penetration of Daylight into Mid-Atlantic and Coastal Waters. *Biol. Bull.*, **65**: 317.

CLARKE, G. L., 1933*b*. Diurnal Migration of Plankton in the Gulf of Maine and its Correlation with Changes in Submarine Irradiation. *Biol. Bull.*, **65**: 402.

CLARKE, G. L., 1934. Further Observations on the Diurnal Migration of Copepods in the Gulf of Maine. *Biol. Bull.*, **67**: 432.

ESTERLY, C. O., 1911. Calanoid Copepods from the Bermuda Islands. *Proc. Am. Acad.*, **47**: 217.

FAGE, LOUIS, 1933. Migrations Verticales périodiques des Animeaux benthiques litteraux. *Conseil Int. Expl. Mer., Rapports et Procès-Verbaux*, **85**: 60.

NICHOLLS, A. G., 1933. On the Biology of C. finmarchicus. III. Vertical distribution and diurnal migration in the Clyde Sea-Area. *Jour. Mar. Biol. Ass.*, **19**: 139.

WORTHINGTON, E. B., 1931. Vertical Movements of Fresh-water Macroplankton. *Int. Rev. Hydrobiol. and Hydrogr.*, **25**: 394.

DIGESTIVE ENZYMES OF THE PELAGIC COPEPOD, CALANUS FINMARCHICUS

R. M. BOND

(From the Woods Hole Oceanographic Institution,[1] Woods Hole, Mass.)

Yonge (1931), in his review of the "Digestive Processes in Marine Invertebrates and Fishes" states that "Little is known of the digestive enzymes of the Entomostraca. . . ." The somewhat incomplete observations here presented are sufficiently illuminating (I believe) to merit publication at this time, particularly since circumstances force me to postpone, for an indefinite period, carrying the work any further.

The feeding mechanism of the calanoid copepods has been described by Storch and Pfisterer (1925), and, perhaps more correctly, by Cannon (1928). They are filter feeders, and their mechanism is so efficient, according to Naumann (1923), that they can remove particles as small as 1 μ in diameter from the water. They can also ingest much larger particles, breaking up and swallowing large diatoms and crustacean nauplii, etc. (Cf. Dakin (1908), Esterly (1916), Lebour (1922), and Marshall (1924) for material observed in the gut.)

Calanus is so small (2.5–6.5 mm.) that it proved impractical to extract the guts alone. Large numbers of the live copepods (copepodid III to adult stages—mostly copepodid IV and V) were separated from other organisms taken in the tows, strained off on bolting silk, and placed in a calibrated test-tube. To each cubic centimeter of copepods was added 0.6 cc. of sea water, 0.4 cc. of 95 per cent alcohol, 1 cc. of toluene, and some glass beads or well washed sand. The tube was stoppered with a rubber stopper (previously boiled in a solution of NaOH and well washed) and shaken violently in a mechanical shaker for an hour or more, until the copepods were reduced to a pink emulsoid. The extraction was then allowed to continue for about 36 hours, and the extract filtered off through filter paper. More toluene was added, and the extract kept at 5–6° C. till used (1 to 7 days). Before use the extract was mixed with an equal volume of buffer. The buffers were made according to the directions given in Clark (1928). Those of pH 1.0, 1.5, and 2.0 were HCl-KCl buffers. For pH 2.4, 2.8, 3.2, 2.6, 4.0, 4.4, 4.8, 5.2, 5.6, and 6.0 the phthalate series was used. For pH 6.4, 6.8, 7.0, 7.2, 7.6, and 8.0 the phosphate series was employed; and for pH 8.49, 8.84, 9.61, 10.96, 11.45, and 12.54 the glycine series.

[1] Contribution No. 56.

These buffers were used in preference to others because it was known that they have no toxic effect on enzymes.

Since the extract itself was at pH 7.0–7.6, and since it is fairly well buffered, it is probable that the pH of the extract-buffer mixtures in the acid range was actually somewhat higher, and those in the alkaline range somewhat lower than the figures given.

No method was devised for accurately determining the pH of the gut of the living copepods. Neutral red colors the lumen so slightly and the gut is so narrow, that it is impossible, without adequate standards, to be certain that the color observed in the mid-gut was actually (as it appeared) the yellowish red of a somewhat alkaline solution, rather than very dilute brick or rose red of neutral or acid conditions. In the decapods (according to the data summarized by Yonge), for which the stomach pH is known, the normal conditions are acid (5.1–5.6), but it would be unsafe to generalize about other Crustacea from this. It may be said that there is nothing known about gut conditions in general, nor about the enzymes of *Calanus* (as will be seen below) to indicate that the gut pH might not theoretically lie anywhere in a range of from at least as low as 6.0 to at least as high as 8.0.

In the following experiments controls, using buffer alone, and buffer with extract kept 10 minutes in a tube in a boiling water bath, were used except where otherwise noted. The four lots of extract used were checked against each other and found to vary somewhat in strength, but were otherwise similar. Each observation given is based on at least two independent experiments—usually three to six.

Proteinase

The method used was that of Pickford and Dorris (1934). Digestion was found to occur from pH 2.4 to 12.54. Above pH 10.96 there was some alkaline hydrolysis of the substrate, so that it is difficult to assign a value to the action of the extract. Below 2.4 there was acid hydrolysis, but the effect on the gelatin was enough unlike that of the enzyme to make a clear differentiation. The speed of digestion at the various pH values is shown in Fig. 1. It will be noted that there is a peak at pH 3.6–4.0 and a much greater one at 8.0–8.49. The " speed of digestion " as shown in the figure is the reciprocal of the logarithm of the time for complete digestion by the most active lot of extract. As this is only comparative, no numerical scale is given.

Amylase

The method of Pickford and Dorris was used for this enzyme also, cornstarch being the substrate. The complete range of pH values was not tested, but an optimum was found at about 7.2, with a very rapid falling off of activity at 6.0 and a less rapid falling off at 8.0.

Lipase

The ethyl butyrate method previously used by me (1933) was adopted, but without the addition of water. Under the conditions of the experiment, the litmus turned pink only after a few days, but by the end of a week, the difference between the experimental and control tubes was extremely striking.

Alginase

Dr. Selman A. Waksman and his associates have become interested in the polymannuronic anhydride known as alginic acid. This (principally in the form of Ca and Mg salts) may make up 20–30 per cent of the air-dried weight of various marine algæ, such as *Fucus, Sargassum,*

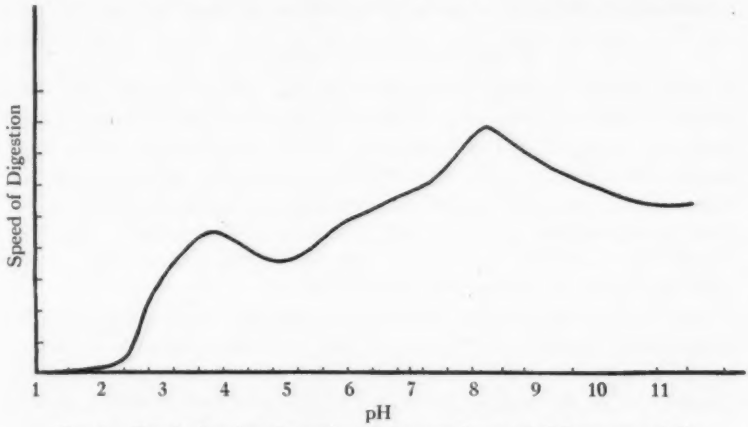

FIG. 1. Graph showing the action of the proteinase of *Calanus* on gelatin.

etc., and it unquestionably is a very important fraction of the total production of marine plants, corresponding to the stored carbohydrates of terrestrial plants. Of course these larger algæ are not ingested by *Calanus* (except perhaps in the form of fine detritus), but their composition may possibly give some indication of the makeup of the unicellular forms. In any case, it was considered desirable to attempt to discover an alginase in *Calanus*. Dr. Waksman kindly provided me a supply of alginic acid, and also a strain of alginic-acid-digesting bacteria, *Bacterium alginovorum* [Cf. Waksman, Carey, and Allen (1934)].

Agar plate methods were found quite unsuited for the purpose, because neither calcium alginate nor alginic acid remains unchanged by the buffers alone near the neutral point. In dry plates, moreover, the acid becomes practically invisible, and no specific stain is known to me for alginic acid, or its breakdown products.

A set-up was made, however, of 2 cc. of 3 per cent faintly alkaline sodium alginate solution, 1 cc. of buffer at pH 8.0, and 1 cc. of *Calanus* extract. A control with boiled extract was used. Two cc. of the liquid was allowed to drop from a pipette with a finely drawn-out tip, and the meniscus timed between marks with a stop-watch. There was not the slightest detectable change in viscosity during a period of 18 days.

The relative strengths of the enzymes investigated cannot be surely told, because the methods used are not comparable. However, a very dilute extract prepared in a preliminary experiment gave good starch digestion in 20–30 minutes at pH 7.0, whereas no digestion of gelatin occurred at any pH during 16 hours. From this it would certainly appear that the carbohydrate-splitting enzyme is much more vigorous than the proteinase—at least in the latter's action on gelatin.

Digestion of Whole Organisms

Suspensions of living *Nitzschia closterium* (Plymouth strain), *Dunaliella salina,* and *Bacterium alginovorum* were placed in drops of enzyme extract at pH 8.0 in depression culture dishes of soft glass, and the liquid covered by a layer of toluene (which, of course, killed the organisms). The culture dishes were kept at room temperature in Petri dishes containing water and toluene, and the toluene over the extract and in the Petri dishes was renewed from time to time. The controls were in the buffer without extract.

Some of the observations cited above show that *Calanus* guts often contain large numbers of diatoms. *Nitzschia* may be taken as a representative of this group of algæ (though not in itself an important constituent of *Calanus* food). In 36 hours the diatoms in the buffer alone appeared almost normal and stained well—practically like living organisms. In the enzyme, however, the tests appeared empty except for a minute refractive body in the center which took methylene blue extremely lightly—the rest of the diatoms remaining unstained.

The green flagellates after 36 hours were badly broken down both in the enzyme and the control, though those in the enzyme appeared slightly more disintegrated. But, after only 20–24 hours, there was a distinct difference: most of those in the control appearing approximately normal, whereas in the extract, although the chloroplast and other cell inclusions were not noticeably changed, they were crowded more or less into one end of the cell, leaving large clear areas in which no cytoplasm could be made out either without or with methylene blue staining.

The bacteria in the buffer control had settled in 36 hours to the bottom of the drop, and formed a slimy mass, much as they do when alive. In the enzyme the bacteria were perceptibly reduced in numbers (by

inspection, to about one-fourth of the control) and were perfectly separate and non-slimy. The individuals remaining, however, took methylene blue very much as the controls did.

Conclusions

Calanus finmarchicus contains enzymes which digest protein, starch, and fat. It has not been shown that these enzymes occur in the lumen of the gut, but that they do is certainly not improbable. Proteolytic digestion is performed by the extracted enzymes from pH 2.4 to above pH 11.0; there is a peak of activity at pH 3.6–4.0, and a still greater peak at pH 8.0–8.49.

Digestion of starch is performed by the extract from slightly below pH 6.0 to above pH 8.0, with an optimum at about 7.2. There is no digestion of alginic acid at pH 8.0, and no evidence was obtained that it is digested at any other pH.

The copepod extract will digest diatoms, bacteria, and at least a part of the cell contents of a green flagellate.

I wish to tender my thanks to the staff of the Woods Hole Oceanographic Institution for the grant of a summer fellowship, and for much invaluable advice and assistance in this work.

BIBLIOGRAPHY

Bond, R. M., 1933. A Contribution to the Study of the Natural Food-cycle in Aquatic Environments etc. *Bull. Bingh. Ocean. Coll.*, **4** (4): 1.

Cannon, H. G., 1928. On the Feeding Mechanism of the Copepods Calanus finmarchicus and Diaptomus gracilis. *Brit. Jour. Exper. Biol.*, **6**: 131.

Clark, W. M., 1928. Determination of Hydrogen Ions. 3d ed., Williams and Wilkins, Baltimore, 1928.

Dakin, W. J., 1908. Note on the Alimentary Canal and Food of the Copepoda. *Int. Rev. ges. Hydrobiol. u. Hydrogr.*, **1**: 772.

Esterly, C. O., 1916. The Feeding Habits and Food of Pelagic Copepods and the Question of Nutrition by Organic Substances in Solution in the Water. *Univ. Cal. Pub. Zool.*, **16**: 171.

Lebour, Marie V., 1922. The Food of Plankton Organisms. *Jour. Mar. Biol. Ass.*, **12**: 644.

Marshall, Sheina, 1924. The Food of Calanus finmarchicus during 1923. *Jour. Mar. Biol. Ass.*, **13**: 473.

Naumann, E., 1923. Spezielle Untersuchungen über die Ernährungsbiologie des tierischen Limnoplanktons. II. Über den Nahrungserwerb der Copepoden and der Rotiferen des Limnoplanktons. *Lund. Univ. Arsskr.*, (2) **19** (6).

Pickford, Grace E., and Frances Dorris, 1934. (An article on micro methods for the detection of digestive enzymes.) *Science* (in press).

Storch, O., and O. Pfisterer, 1925. Der Fangapparat von Diaptomus. *Zeitschr. vergl. Physiol.*, **3**: 330.

Waksman, S. A., Cornelia L. Carey, and M. C. Allen, 1934. Bacteria Decomposing Alginic Acid. *Jour. Bact.* (in press).

Yonge, C. M., 1931. Digestive Processes in Marine Invertebrates and Fishes. *Jour. Cons. Int. Expl. Mer.*, **6**: 175.

THE DEVELOPMENTAL STAGES OF THE COPEPOD EPILABIDOCERA AMPHITRITES McMURRICH

MARTIN W. JOHNSON

(From the University of Washington Oceanographic Laboratories, Seattle, Washington)

Introduction

Considerable importance is attached to the study of the larval stages of copepods for two reasons: (1) from a taxonomic standpoint the type of larva may throw light on the natural relationship of the adult animals; (2) for the most effective study of the importance of copepods in the economy of the sea it is necessary to take into consideration not only the adult animals but also to distinguish the developmental stages and ascertain the habits and requirements of each.

Epilabidocera amphitrites is a Pacific species occurring on the west coast of North America from whence it was first described by McMurrich (1916) from submature specimens (fifth copepodid) taken off Amphitrite Point, Vancouver Island. The original description was given under the generic name *Paralabidocera,* but Wilson (1932) has pointed out that this name was preoccupied and has therefore proposed the generic name here employed. Adult descriptions are given by Willey (1920) and Esterly (1924).

This species has been reported by Willey (1920) from the northwest coast of Alaska and the Gulf of Alaska, and by Esterly (1924) from San Francisco Bay. As a rule it has not been taken in large numbers but Esterly found it in swarms in San Francisco Bay. Spring swarms also occur at Friday Harbor, Washington.

The writer wishes to thank Dr. T. G. Thompson, Director of the University of Washington Oceanographic Laboratories, for laboratory facilities.

Procedure

In studying the successive nauplius stages, the specimens were taken directly from the plankton at Friday Harbor, and identification of the species was accomplished by selecting specimens of the sixth nauplius stage from fresh plankton hauls and rearing them in section jars through the critical moult to the first copepodid stage. This stage is easily linked to the second copepodid stage, and the following stages are even more readily referable to the adult. Aside from size and pig-

mentation, the presence of dorsal cuticular lenses and, following the first copepodid stage, the rostral processes and cephalic hooks serve as unmistakable characters identifying the stages with *Epilabidocera amphitrites* which is the only typical species of Pontellidæ that has been found in this immediate region.

All drawings were made with the aid of a camera lucida.

The Nauplius Stages

After the first nauplius stage, the long, slender and straight body with its long caudal spine and the long slender first antennæ make the larvæ of *Epilabidocera amphitrites* conspicuous among the many nauplii occurring in this area. In the living condition, the first antennæ are directed straight forward, and when at rest are in contact practically their whole length. The end segment of these appendages is orange-brown in color and this pigment is also found in the posterior end of the body and to a lesser degree on the ventral side in front of the mouth. The anterior end of the carapace is bent sharply downward forming a curved margin which is slightly constricted on each side of the head. The eye is dark brown and the labrum is long and of medium width. The long bristles occurring on the appendages are plumose but often the fine processes can be discerned only with difficulty. These fine processes have been omitted in the figures except in a few instances where it appeared significant that they be included.

Only a few specimens of Stage I were found and these were all taken from preserved material, hence it is not possible to state with certainty that the pigmentation, etc., described from the living condition for the older specimens applies equally to the first stage.

Stage I (Fig. 1)

Body.—0.182–0.192 mm. (average 0.188 mm.) long, oval with the anterior end slightly the broader, posterior end armed with two straight spines of equal length.

First Antenna.—Three segments, the first short and with one short ventral bristle; the second short and bearing ventrally one very short and one long bristle; the third or distal segment bearing three long terminal bristles and ventrally near the tip a short blunt bristle; dorsally there are two very fine smooth bristles and on the inner margin posterior to the middle there is situated a transverse series of very minute spines.

Second Antenna.—First basipod with one masticatory hook. Second basipod with one masticatory hook, an adjacent small outwardly directed spine and one small spine near the distal end. Endopod of one segment with two long terminal bristles and one long lateral bristle.

Exopod of six segments, the first short with no bristle, 2–5 with one bristle each, and the sixth with two bristles.

Mandible.—First basipod with a small chewing process bearing a small spine. Second basipod with two spines on the inner side. Endopod of two completely fused segments, the first with two weak spines and the second with one short and two long bristles. Exopod of four segments, 1–3 with one bristle each, the fourth with two bristles.

Stage II (Fig. 2)

Body.—0.253–0.274 mm. (average 0.264 mm.) in length, slender, evenly tapered posteriorly and terminating in one long heavy setose spine and, to the right of it, one shorter dorsally directed plumose bristle. At the base of each of these there are a number of very short spines. Two series of similar short spines also appear on each side of the body in the posterior lateral region (Fig. 2, *ls*).

First Antenna.—The first and second segments as in Stage I; the distal segment bears at the tip three long bristles and one shorter accessory bristle, and near the tip there are two fine bristles on the dorsal and two on the ventral margin. Each margin also bears a separate row of fine hair-like bristles. The inner transverse series of minute spines as in I.

Second Antenna.—First basipod with one masticatory hook and a small outwardly directed spine near the base of the hook. Second basipod as in I. Endopod with two long terminal bristles, and on the lateral margin, the one long bristle of Stage I is replaced by one short and two long hair-like bristles and an adjacent series of minute bristles. Exopod as in I but with two bristles on the second segment.

Mandible.—First and second basipods as in I. Endopod as in I but with three strong hooked spines on the first segment and two long

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PLATE I

Epilabidocera amphitrites

FIG. 1. Nauplius Stage I.
FIG. 2. Nauplius Stage II.
FIG. 3. Nauplius Stage III.
FIG. 4. Nauplius Stage IV.

Abbreviations:
a—first antenna.
a2—second antenna.
en—endopod.
ex—exopod.
l—labrum.
ls—series lateral spines.
m—mandible.
mx—first maxilla.
mx2—second maxilla.
vh—ventral hooks.

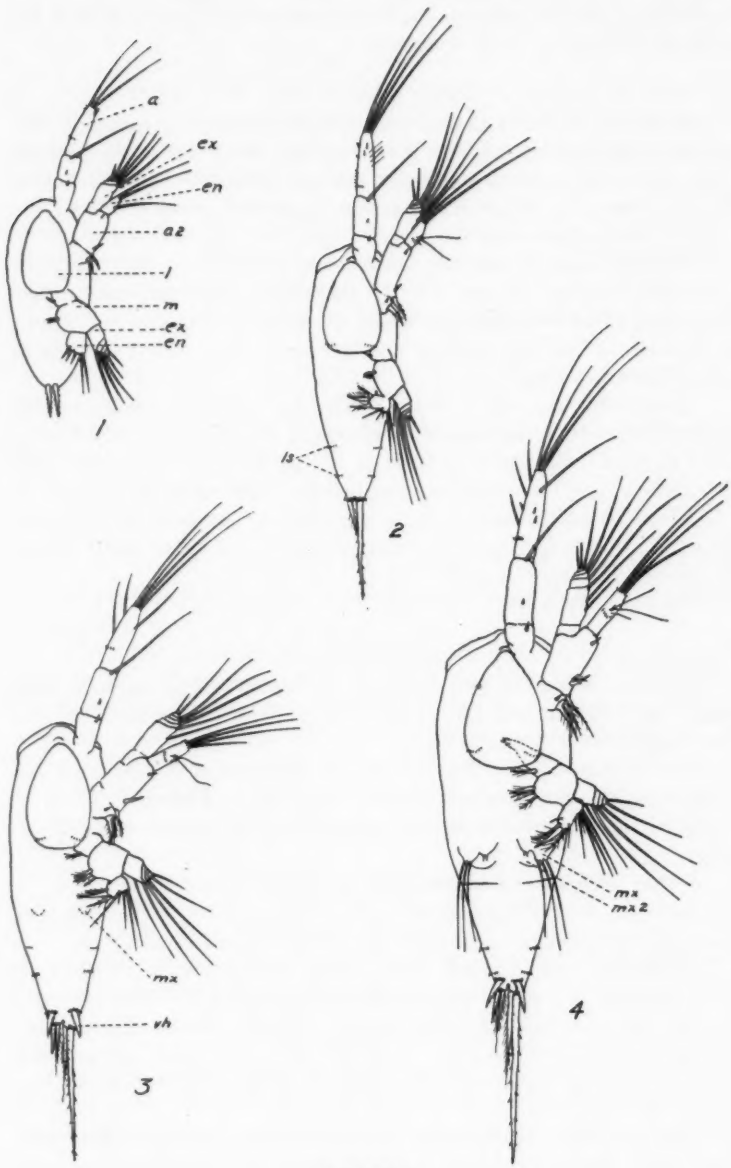

PLATE I

and three shorter bristles on the second segment. Exopod as in I but with two bristles on the first segment.

Stage III (Fig. 3)

Body.—0.312–0.361 mm. (average 0.337 mm.) long, posterior end armed with one long and one short terminal setose spine and between these two plumose bristles, and just anterior to them one pair of ventral hooks. Only one series of minute spines occurs on each side of the body in the posterior lateral region.

First Antenna.—Segments one and two as in II; the distal segment with four terminal bristles as in II, two long dorsal marginal bristles, one long ventral marginal bristle and an inner transverse series of minute spines. The fine hair-like bristles found on the distal segment in stage II are wanting.

Second Antenna.—First basipod with one strong masticatory hook, a short hook placed upon its base and a small outwardly directed spine near its base. Second basipod as in II. Endopod as in II but with one medium and three long terminal bristles. Exopod as in II.

Mandible.—First basipod with the chewing process somewhat enlarged and bearing a spine. Second basipod with three inner spines. Endopod and exopod as in II.

First Maxilla.—Bud (?).

Stage IV (Fig. 4)

Body.—0.380–0.412 mm. (average 0.402 mm.) long, posterior end armed as in III but with two series of minute spines on each side of the body in the posterior lateral region.

First Antenna.—The first and second segments as in III; the distal segment with four terminal bristles, one short and three long dorsal marginal bristles and a transverse series of minute spines on the inner side.

Second Antenna.—First basipod as in III. Second basipod as in III but with an additional short spine. Endopod as in III. Exopod as in III but with three bristles on the second segment.

Mandible.—First basipod with a long, strong chewing process or blade toothed at the end and bearing a spine near the base. Second basipod with one weak and three stronger spines. Endopod with three strong hooked spines and two weak bristles on the first segment and four long and two short very weak bristles on the second segment. Exopod as in III.

First Maxilla. Rudimentary with two lobes, the outer lobe with three long weak bristles and the inner lobe with two very short weak bristles.

Second Maxilla.—Bud.

Stage V (Fig. 5)

Body.—0.421–0.500 mm. (average 0.481 mm.) long, posterior end armed as in IV but with the addition of a pair of lateral hooks similar to the ventral pair. Just anterior to the lateral hooks there remains but one series of minute spines on each side of the body.

First Antenna.—Segments 1 and 2 as in IV; the distal segment with four terminal bristles, four long and two short dorsal marginal bristles, one long and two short ventral marginal bristles and a transverse series of minute spines on the inner side.

Second Antenna.—As in IV but with five terminal bristles on the endopod.

Mandible.—As in IV but with the addition of one very weak bristle on the second basipod.

First Maxilla.—Rudimentary, the outer and inner lobe with five and two long bristles respectively.

Second Maxilla.—Bud.

Maxilliped.—Bud.

Stage VI (Fig. 6)

Body.—0.540–0.575 mm. (average 0.564 mm.) long, posterior end armed as in V.

First Antenna.—The first and second segments as in V; the distal segment with four terminal bristles, five long and three short (alternating with the long) dorsal marginal bristles, one long and four short ventral marginal bristles and a transverse series of minute bristles on the inner side.

Second Antenna.—As in V but the endopod with four hair-like bristles and a group of very fine short bristles on the lateral margin.

Mandible.—As in V.

First Maxilla.—Rudimentary, the outer and inner lobe with seven and four bristles respectively.

Second Maxilla.—Bud.

Maxilliped.—Bud.

First and Second Feet.—Rudimentary.

The Copepodid Stages

The sixth nauplius stage terminates in the critical moult from which the first copepodid emerges in the form of the adult but lacking in certain characters relating to the number of appendages, segmentation, etc. There is a total of six copepodid stages, the last of which is the adult

animal. Throughout these stages the animal remains relatively slender and tapered at the anterior end. In the living condition the urosome and caudal rami are orange-brown in color, the pigment usually extending into the caudal bristles. It is also present in the distal segment of the first antenna in the younger stages (and in the right antenna of the adult male) and in the anterior part of the head, extending in increasing intensity from behind the cuticular lenses towards the rostrum.

Stage I (Figs. 7 and 8)

Body Length.—To end of caudal rami 0.76–0.82 mm., thorax [1] of four segments, abdomen of one segment, dorsal cuticular lenses present but the rostral processes and lateral cephalic hooks which are typical of the later stages are wanting. The anterior end of the head is flexed downward forming a blunt rounded knob-like rostrum. The urosome and caudal rami are symmetrical.

First Antenna.—Ten segments.

Second Antenna.—First basipod with one bristle. Second basipod with two bristles. Endopod of two segments, the first with two bristles; the second forming two lobes, the inner lobe with three long and one shorter bristle, the outer lobe with five long and one shorter bristle. Exopod of five segments, the first short and bearing one bristle, the second long and with two lateral and two distal bristles, the third and fourth with two bristles each and the fifth with two terminal bristles.

Mandible.—Mandibular blade with five teeth; palp with a short first basipod, long second basipod and two short rami, the inner ramus with a terminal group of four bristles and a lateral group of four bristles, the outer ramus with six bristles.

[1] The thorax of the adult copepod is considered to consist of six segments. In my previous paper (Johnson, 1934) the term 'urosome' is applied to that portion of the body posterior to the last thoracic segment bearing visible feet. The term 'thorax' used in explanation of Figs. 2–6, Plate III, should read 'metasome.'

PLATE II

FIG. 5. Nauplius Stage V.
FIG. 6. Nauplius Stage VI.
FIG. 7. Copepodid Stage I, lateral.
FIG. 8. Copepodid Stage I, dorsal.

Abbreviations:
f—first feet.
f2—second feet.
lh—lateral hooks.
mp—maxilliped.
mx—first maxilla.
mx2—second maxilla.

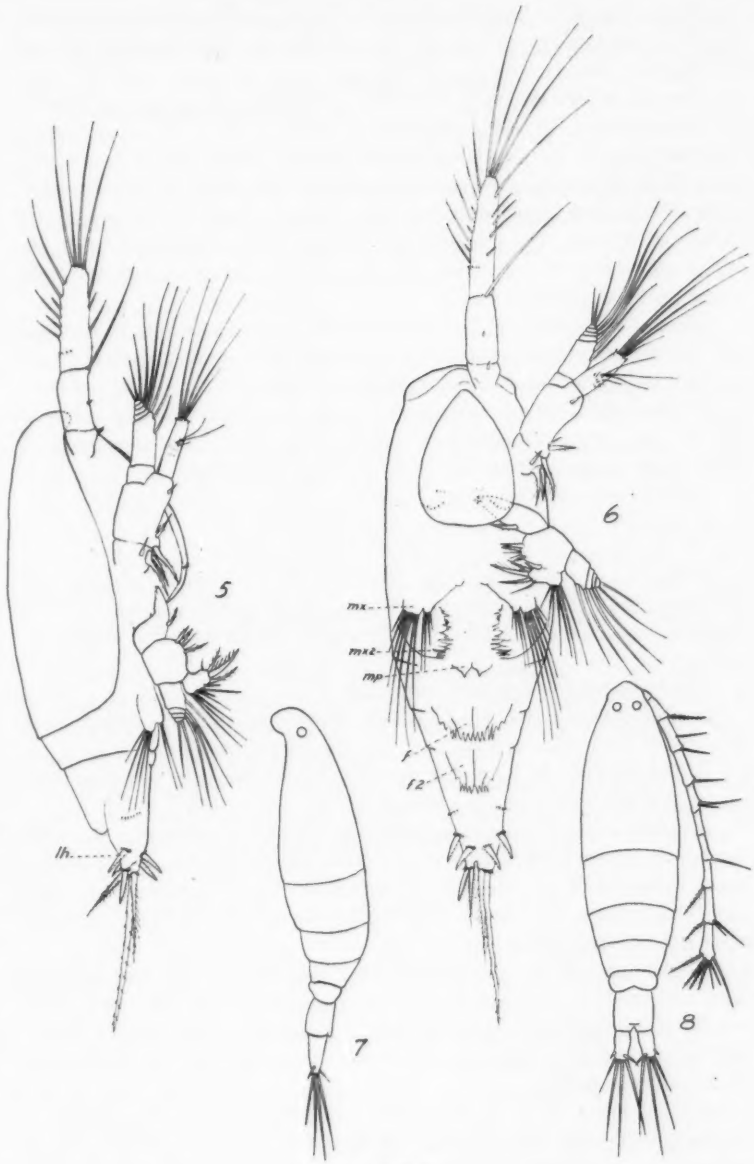

PLATE II

First Maxilla.—All lobes formed as in the adult but each with fewer bristles except the inner lobe of segment two and segment three, each of which bears three bristles as in the adult.

Second Maxilla.—As in the adult with fifteen long, strong bristles.

Maxilliped (Fig. 10).—Uniramose with a total of four segments; the first lobe of the first basal with one short setose bristle, the second lobe of the first basal with one short and one long setose bristle, the third lobe of the first basal with one long setose bristle and a very small bristle at its base; the second basal with one distal plumose bristle; the third segment with one distal plumose bristle; the distal segment with three terminal smooth bristles.

First Feet.—First and second basipods with no bristles. Endopod of one segment with six bristles. Exopod of one segment with four outer spines, a terminal blade, and three bristles.

Second Feet.—First and second basipods with no bristles. Endopod of one segment with six bristles. Exopod of one segment with three outer spines, a terminal blade, and three bristles.

Third Feet.—Rudimentary.

Stage II

Body.—1.01–1.16 mm. long, thorax of five segments, abdomen of one segment; dorsal cuticular lenses, rostral processes and lateral cephalic hooks present (Fig. 9). Urosome and caudal rami symmetrical.

First Antenna.—Fourteen segments.

Second Antenna.—As in I but with three long and two shorter bristles on the inner lobe of the distal segment of the endopod.

Mandible.—As in I but the inner branch of the palp with five long and two short bristles in the terminal group.

Maxilliped.—A total of five segments; the first basal as in I but with two short setose bristles on the first lobe; the second basal with a serrated margin and one distal plumose bristle; the third and fourth segments each with one distal plumose bristle and the fifth with three smooth terminal bristles.

First Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of two segments, the first with one inner bristle, the second with seven bristles. Exopod of two segments, the first with one outer thorn, the second with three outer thorns, a terminal blade and four bristles.

Second Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of one segment with seven bristles. Exopod of two segments, the first with one outer thorn, the second with two outer thorns, a terminal blade and four bristles.

Third Feet.—First and second basipods with no bristles. Endopod of one segment with six bristles. Exopod of one segment with three outer thorns, a terminal blade, and three bristles.

Fourth Feet.—Rudimentary.

Stage III

Body.—1.43–1.50 mm. long, thorax of six segments, abdomen of one segment. Cuticular lenses, rostral processes and cephalic hooks present. Posterior end of body symmetrical.

First Antenna.—Sixteen segments.

Second Antenna.—As in II.

Mandible.—As in II.

Maxilliped.—A total of six segments, otherwise as in II.

First Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of two segments, the first with one inner bristle, the second with eight bristles. Exopod of two segments, the first with one outer thorn and one inner bristle, the second with three outer thorns, a terminal blade and four bristles.

Second Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of one segment with nine bristles. Exopod of two segments, the first with one outer thorn and one inner bristle, the second with three outer thorns, a terminal blade and five bristles.

Third Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of one segment with seven bristles. Exopod of two segments, the first with one outer thorn, the second with two outer thorns, a terminal blade and four bristles.

Fourth Feet.—First and second basipods with no bristles. Endopod of one segment with six bristles. Exopod of one segment with three outer thorns, a terminal blade, and three bristles.

Fifth Feet.—Rudimentary.

Stage IV

Body.—2.23–2.35 mm. long, thorax of six segments (the fifth thoracic segment symmetrical with slightly pointed corners), abdomen of two segments in both sexes.

First Antenna.—Twenty-three to twenty-four segments.

Second Antenna.—As in III but with an additional bristle in each of the two lobes of the terminal segment of the endopod.

Mandible.—As in III.

Maxilliped (Fig. 11).—As in III but with one very small bristle at the base of the long bristle on the second lobe of the first basal and two

very small bristles at the base of the long bristle on lobe three; basal two has three distal plumose bristles and segment three has two distal plumose bristles.

First Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of two segments, the first with one inner bristle, the second with eight bristles. Exopod of two segments, the first with one outer thorn and one inner bristle, the second with three outer thorns, a terminal blade and four bristles.

Second Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of one segment with ten bristles. Exopod of two segments, the first with one outer thorn and one inner bristle, the second with three outer thorns, a terminal blade, and five bristles.

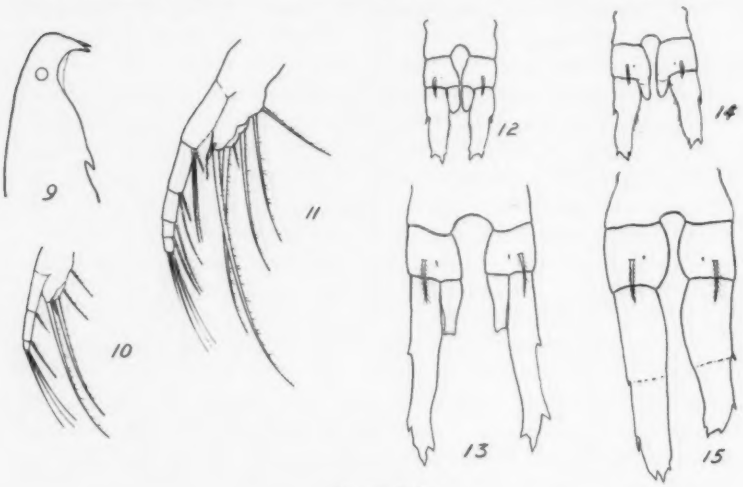

PLATE III

FIG. 9. Head copepodid Stages II–VI, lateral.
FIG. 10. Maxilliped copepodid Stage I.
FIG. 11. Maxilliped copepodid Stage IV.
FIG. 12. Fifth feet copepodid Stage IV, female.
FIG. 13. Fifth feet copepodid Stage V, female.
FIG. 14. Fifth feet copepodid Stage IV, male.
FIG. 15. Fifth feet copepodid Stage V, male.

Third Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of one segment with nine bristles. Exopod of two segments, the first with one outer thorn and one inner bristle, the second with three outer thorns, a terminal blade and five bristles.

Fourth Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of one segment with eight bristles. Exopod

of two segments, the first with one outer thorn, the second with three outer thorns, a terminal blade and five bristles.

Fifth Feet, Female (Fig. 12).—Symmetrical, biramose, the inner ramus being short and rounded at the tip, the outer ramus of one segment with one outer thorn and one small and two large spines at the tip, and on the inner margin a number of fine hairs. The posterior surface of the second basipod bears one long and one very short bristle.

Fifth Feet, Male (Fig. 14).—Symmetrical, biramose, the inner ramus short and rounded as in the female, the outer ramus as in female but with two large and two very small spines at the tip; second basipod with bristles as in the female.

Stage V

Body.—2.73–3.30 mm. long, thorax of six segments, the fifth thoracic segment asymmetrical in the male due to increased length of the right corner forming a conspicuous point; abdomen symmetrical and of two segments in the female and three in the male.

First Antenna.—Twenty-four segments.

Second Antenna.—As in IV but with seven and eight bristles respectively in the inner and outer lobes of the distal segment of the endopod.

Mandible.—As in IV.

Maxilliped.—As in IV.

First Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of three segments, the first with one inner bristle, the second with two inner bristles, the third with six bristles. Exopod of three segments, the first and second each with one outer thorn and one inner bristle, the third with two outer thorns, a terminal blade and five bristles.

Second Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of two segments, the first with three inner bristles, the second with eight bristles. Exopod of three segments, the first and second each with one outer thorn and one inner bristle, the third with three outer thorns, a terminal blade and five bristles.

Third Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of two segments, the first with three inner bristles, the second with eight bristles. Exopod of three segments, the first and second each with one outer thorn and one inner bristle, the third with three outer thorns, a terminal blade and five bristles.

Fourth Feet.—First basipod with one inner bristle. Second basipod with no bristle. Endopod of two segments, the first with three inner bristles, the second with seven bristles. Exopod of three segments, the

first and second each with one outer thorn and one inner bristle, the third with three outer thorns, a terminal blade and five bristles.

Fifth Feet, Female (Fig. 13).—Symmetrical, biramose, the inner ramus short and with two short points, the outer ramus of one long segment with one outer spine near its middle and one inner and one outer spine near the one terminal spine; second basipod with bristles as in IV.

Fifth Feet, Male (Fig. 15).—Asymmetrical, uniramose, each foot consisting of first and second basipods with bristles as in the female, and two firmly fused segments of the exopod. Distally the first segment has an outer spine, the second segment bears one outer lateral spine, two terminal spines, and one very small inner spine near the tip.

Stage VI, Adult

Discussion

Epilabidocera amphitrites possesses a typical calanoid nauplius larva. The body shape resembles most closely the larvæ of *Pontella mediterranea, Centropages hamatus, Acartia longiremis* and *Tortanus discaudatus* in having a straight hind body which is not sharply marked off from the fore body as occurs in *Calanus finmarchicus* and *Temora longicornis*. A comparison with the incomplete figures of Claus (1893, Plate I) indicates a striking similarity to the late nauplius of *P. mediterranea*. In both species the body is unusually slender and the caudal armature is apparently of the same type. The caudal armature also recalls that of *C. hamatus* and *T. longicornis* in the presence of a long heavy left terminal spine. In Stage II this likeness to the corresponding stage is greater in the former species, whereas the following stages recall more nearly the larva of the latter. In the latter species, however, the short right spine partakes less of the character of a "hook" and there is a maximum of three pairs of lateral hooks and two pairs of ventral hooks as contrasted with one pair each in *E. amphitrites*. Another similarity is seen in the sixth stage when both species bear eight dorsal marginal bristles and five ventral marginal bristles on the distal segment of the first antenna, but *E. amphitrites* is unique in having an alternation of long and short bristles on the dorsal margin.

The likeness of the caudal armature to that of *C. hamatus* may be said to diminish after the second stage, and yet this dissimilarity is not so great if the end spines ("Endborsten") which appear in the third stage of *C. hamatus* (Oberg, 1906, Plate II, Fig. 3) can be considered distally placed lateral hooks, as they appear to be.

Table I gives a brief summary of the characters useful in identification of the successive nauplius stages of *E. amphitrites*.

TABLE I

Identification Table for Nauplius Stages of Epilabidocera Amphitrites

Stage No.	I	II	III	IV	V	VI
Length in mm.	0.182–0.192	0.253–0.274	0.312–0.361	0.380–0.412	0.421–0.500	0.540–0.575
First antenna distal segment	3 terminal bristles	4 terminal bristles, 1 dorsal and 1 ventral series of fine hairs	4 terminal bristles, fine hairs wanting, 1 ventral and 2 dorsal marginal bristles	As in III, but 4 dorsal and 3 ventral marginal bristles	As in IV, but 6 dorsal and 3 ventral marginal bristles	As in V, but 8 dorsal and 5 ventral marginal bristles
Caudal armature	2 equal end spines	1 long end spine, 1 shorter end bristle, 2 pairs series minute lateral spines	1 long and 1 short end spine, 2 end bristles, 1 pair ventral hooks, 1 pair series minute lateral spines	As in III, but with 2 pairs series minute lateral spines	As in IV, but also 1 pair lateral hooks and only 1 pair series minute lateral spines	As in V
First maxilla	0	0	Bud?	Rudimentary	Rudimentary	Rudimentary
Second maxilla	0	0	0	Bud	Bud	Rudimentary
Maxilliped	0	0	0	0	Bud	Bud
First and second feet	0	0	0	0	0	Rudimentary

The copepodid stages follow the regular order of development. In the development of the feet, it will be noted that upon first appearance (after the rudimentary stage) each ramus of each pair of feet possesses but one segment. With the next moult the exopod (and also the endopod in the first pair) divides once and remains thus through the subsequent moults to the fifth stage, when each branch of all the feet (excepting the fifth pair) add one more segment, giving the total number found in the adult. This was also the method of development of the first four pairs of feet in *Tortanus discaudatus* (Johnson, 1934). In *Calanus finmarchicus* the development is similar to this except that the moult immediately following the appearance of the feet gives rise to two segments in both rami of each pair of feet. After this they remain unchanged until the fifth stage when the adult condition is acquired by the addition of one more segment to each branch (Lebour, 1916, Plate V). *Centropages* evidently also follows this routine (Oberg, 1906, Plate VII, Fig. 7, *A–F*).

In the sixth stage of *E. amphitrites* the fifth feet of the male undergo drastic modification upon reaching maturity. It is of interest to note that in the fourth copepodid stage the fifth feet are practically identical in the sexes, both possessing a short inner ramus. There is a slight difference relative to the terminal spines of the exopod. In this stage the sexes can be distinguished best by the slightly swollen right antenna of the male. In the fifth stage the male has completely lost the inner ramus, whereas in the female it has developed somewhat but still remains rudimentary. The appearance and subsequent disappearance of the inner ramus in the male suggests a stronger tendency in the female to retain the primitive character in this respect.

Up to the adult stage the urosome is symmetrical in both sexes. The only obvious asymmetry in the body in the fifth stage is found in the fifth thoracic segment of the male, which is drawn out to a longer point on the posterior margin of the right side. In the adult the urosome is very asymmetrical, particularly in the female.

The amount of asymmetry found in the nauplius stages of some copepods may not be especially significant in indicating the extent of asymmetry present in the adult. Adult asymmetry comes mainly in the sixth or adult stage and appears to be closely associated with sexual maturity. It must be said, however, that where marked asymmetry occurs in the nauplius stages, some type of body asymmetry can be expected in the adult animal or in one or more species within the family or genus to which it belongs, but not necessarily to the same species. For example, the nauplius stages of *Temora longicornis, Centropages hamatus,* and *Epilabidocera amphitrites* are strikingly asymmetrical, and

TABLE II

Identification Table for Copepodid Stages of E. amphitrites

Stage No.	I	II	III	IV	V	VI
Length in mm.	0.76–0.82	1.01–1.016	1.34–1.50	2.23–2.35	2.53–3.30	3.2–4.0
First antenna	10 segments	14 segments	16 segments	23–24 seg. right in male slightly swollen	24 seg. right in male swollen	24 seg. right in male geniculated
Feet present	1st and 2d (3d rudimentary)	1st, 2d and 3d (4th rudimentary)	1st, 2d, 3d and 4th (5th rudimentary)	1st, 2d, 3d, 4th and 5th	As in IV but differ in sexes	As in V
Thorax	4 segments	5 segments	6 segments	6 segments	6 segments	6 segments
Abdomen	1 segment	1 segment	1 segment	2 segments	2 seg. ♀ 3 seg. ♂	Asymmetrical 2 segments 4 segments

of the adults *T. longicornis* is usually symmetrical (but in *T. turbinata* the anal segment is somewhat asymmetrical in the female); *C. hamatus* is slightly asymmetrical in the genital segment and in the posterior corners of the last thoracic segment of the female; and *E. amphitrites* is very asymmetrical. *Tortanus discaudatus* and *Acartia longiremis* possess relatively little asymmetry in the larvæ, while the urosome and caudal rami of the former are very distorted in both sexes in the adult, and only a little tendency to distortion is seen in the caudal rami of the female in the latter.

Calanus finmarchicus and *Pseudocalanus minutus* (syn. *elongatus*) represent the calanoid group with symmetrical bodies when mature and little or no asymmetry in the nauplius stages (Oberg, 1906; Lebour, 1916).

The typical harpacticid nauplius (Gurney, 1930, *Longipedia*, Fig. 10) and adult are symmetrical, and the same is true of the Cyclopoida (Gibbons and Ogilvie, 1933, *Oithona helgolandica* and *O. spinirostris*, Plates I and II).

From the above few comparisons it is seen that, in so far as the life histories of marine copepods have been investigated, there appears to be some tendency to larval asymmetry in the calanoid group in which some adults are asymmetrical. Whether or not there is a period in the early copepodid stages in which the asymmetry remains latent, only additional investigation on life histories will show. It must be borne in mind that the asymmetrical calanoids (for example, the Pontellidæ) usually also possess striking modification in secondary sex characteristics relative to the feet and antenna which are not indicated in the larvæ, and that the body asymmetry may develop in the adult due to the same more recently acquired trend.

Table II gives briefly the most useful characteristics for identification of the copepodid stages of *Epilabidocera amphitrites*.

BIBLIOGRAPHY

Claus, C., 1893. Ueber die Entwicklung und das System der Pontelliden. *Arb. Zool. Inst. Wien u. Zool. Sta. Triest*, **10**: 233.

Esterly, C. E., 1924. The Free-swimming Copepoda of San Francisco Bay. *Univ. Calif. Publ. Zool.*, **26**: 81.

Gibbons, S. G., and H. S. Ogilvie, 1933. The Development Stages of Oithona helgolandica and Oithona spinirostris, with a Note on the Occurrence of Body Spines in Cyclopoid Nauplii. *Jour. Mar. Biol. Ass.*, N. S., **18**: 529.

Gurney, R., 1930. The Larval Stages of the Copepod Longipedia. *Jour. Mar. Biol. Ass.*, N. S., **16**: 461.

Johnson, M. W., 1934. The Life History of the Copepod Tortanus discaudatus (Thompson and Scott). *Biol. Bull.*, **67**: 182.

Lebour, M. V., 1916. Stages in the Life History of Calanus finmarchicus (Gunnerus), Experimentally Reared by Mr. L. R. Crawshay in the Plymouth Laboratory. *Jour. Mar. Biol. Ass.*, N. S., **11**: 1.

McMURRICH, J. P., 1916. Notes on the Plankton of the British Columbia Coast. *Trans. Roy. Soc. Canada,* **10** (Ser. 3, Sect. 5): 75.

OBERG, M., 1906. Die Metamorphose der Plankton-Copopoden der Kieler Bucht. *Wiss. Meeresunters. Komm. wiss. Untersuch. deutsch. Meere in Kiel,* N. F. (Abt. Kiel), **9:** 37.

WILLEY, A. A., 1920. Report of the Canadian Arctic Expedition, 1913–18. Vol. VII. Crustacea. Part K. Marine Copepoda, pp. 1–46k.

WILSON, C. B., 1932. The Copepods of the Woods Hole Region, Massachusetts. *U. S. Nat. Mus. Bull.,* No. 158: 1.

PULSATIONS IN THE NEREIS EGG

LEIGH HOADLEY[1]

(From the Biological Laboratories, Harvard University, and the Marine Biological Laboratory, Woods Hole)

It is a well-known fact that the eggs of many forms change their shape markedly in the period between the time of fertilization and first cleavage. This is more noticeable in the eggs of some animals than in those of others. It is especially evident in such an egg as that of *Chætopterus,* where a polar lobe is formed just prior to the first cleavage. In the eggs of annelids and mollusks it is quite usual for the formation of the polocytes to be accompanied by changes in the gross shape of the egg, which becomes somewhat flattened just before the appearance of the polar elevation.

Movements of the cytoplasm of the central and more peripheral regions have been described in a large number of eggs. Many of these are mass movements which result in the isolation of specific regions. This is well illustrated by the processes which lead to the formation of the yellow crescent of the egg of *Styela* and of the grey crescent of eggs of certain amphibia. Similarly, mass movements and migration of regions have been noted in the developing egg of *Chætopterus* (Lillie, 1906) and the ctenophore (Spek, 1926). Such mass movements are slightly different from those characteristic of the telolecithal eggs of certain fishes and the squid, where the first visible evidence of activation and development comes with the streaming of the cytoplasm and its subsequent accumulation in the blastodisc.

Changes in the gross shape of the egg or of parts of the developing germ may or may not be related to the isolation of specific regions. In the coelenterates, constant movement is not uncommon, the most extensive being found in *Hydra,* which is "amoeboid" during its early development. The deformation of the egg during polar body elevation and that accompanying the formation of the polar lobe are essentially the same, though the former is not immediately related to any developmental restriction. There is still another group of movements involving altered relationships between parts of the cytoplasm. They appear at the time of cell division and are definitely related to cleavage. Such flowing movements are probably of general occurrence and have been

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described and discussed by so many workers that no specific examples need be cited here.

The present paper is a report of changes in the gross shape of the egg of *Nereis* which take place in the interval between sperm attachment and the second cleavage. Though this activity is not apparent on casual observation, it proves to be very extensive when camera lucida drawings of the egg are made at short intervals and compared. The search for indications of activity was prompted by the observation of certain phenomena associated with the division of the cell in the Canti Tissue Culture Film and with the development of the egg of the rabbit as seen in the film by Professor Warren Lewis and Dr. Paul W. Gregory.

The egg of *Nereis* was selected for use in making these observations for two reasons. This egg is inseminated while the germinal vesicle is intact, and hence the period of observation includes not only the time of first and second cleavage but also of maturation. As will be seen below, these periods are of great interest. Moreover, the egg of *Nereis* is enclosed within an easily determined membrane and contains many oil droplets which are constant in their position. Both supply reference points on which one may depend in determining, first, the extent of the deformation of the egg and, second, its constant orientation in the field of the microscope.

The eggs were prepared for observation in the following way. A large female collected the previous night was placed in a finger bowl with an active male. Both were immediately excited and shedding commenced. A stop watch was used for timing observations. Approximately a minute after the first appearance of the eggs, a very few were placed on a slide in a considerable amount of sea water. The cover glass was supported and sealed on three sides with vaseline. Eggs treated in this way complete the first cleavage at the same time as the controls. The first batch of eggs tested was placed on a slide without the precaution of the vaseline seal. Very interesting information was obtained from this and similar experiments which will be considered below.

Inasmuch as but a few of the eggs were placed under the cover glass, not more than two or three at most were included in one field of the microscope and then, often only by the use of the low power. The manipulation and selection of the eggs to be observed required some five minutes so that observations usually began at about that time. While watching the eggs, it is relatively simple to tell in just what regions changes are taking place, for, aside from the oil droplets, the edge of the fertilization membrane gives a reference line to which

changes in shape may be referred. Both oil droplets and membrane were used in continuous observation but their use in making records had to be abandoned. It was soon found that the time required to draw two sets of lines was too great to be practical. Speed was required and hence eggs were drawn in the same orientation without reference lines. Unfortunately, alterations in contour which appear great on observation of the egg within the membrane are not as evident when the outline of the egg alone is examined. In order to minimize any error which might arise from haste and slight inaccuracies in tracing, an initial magnification of 200 diameters was employed and the sketches made by means of a camera lucida. Subsequent photographic reduction has led to more accuracy than could have been hoped for at the magnification given in the text figure. An effort was made to draw the egg at 20 to 30-second intervals up to the time of first cleavage. The number given in each of the accompanying sketches represents the time in minutes and seconds which elapsed between the initiation of shedding by the female and the completion of the sketch. Complete pictorial histories of individual eggs, while a part of the records, are not included here for obvious reasons.

In making observations of this kind it is well to bear in mind several possible sources of error. If the orientation of the egg changes during the course of the observation, no positive information is obtained inasmuch as the turning of the egg may lead to changes in shape which are apparent rather than real. In like manner it is wise to be very cautious in recording swelling and shrinking of the eggs, for these may be the result of modifications in the shape which would be evident if viewed from the side, but which may readily be misinterpreted when seen from above. Both of these effects have been controlled in the present observations.

Observations

The observations described below were made at the Marine Biological Laboratory during two successive summers. The results are similar and so consistent that part of but one series of observations need be considered in any detail. A general statement of the findings will be presented first.

The development of the egg of *Nereis* is so well known that but an outline need be presented here. If the cultures are maintained at a temperature of from 18° to 19° C., the main events which serve for the identification of the various stages occur approximately as seen in Table I. All of the events recorded in the table are separated by appreciable intervals of time save for the final engulfment of the sperm head

and first polar body formation. For this reason the 100-minute period has been subdivided for convenience into three shorter intervals indicated in the table as 'A,' 'B,' and 'C.' The first ('A') covers the time elapsing between the insemination of the eggs and the final incorporation of the sperm head within the cortex of the egg. This includes the breakdown of the germinal vesicle. The second period ('B') begins with the isolation of the first polar body as a discrete unit and ends with the elevation of the second polar protuberance. In reality the period stops just before the elevation of the cytoplasm but the egg must be followed to that stage before the exact degree of development can be determined. The third interval ('C') covers the period from the complete isolation of the second polar body up to the time of the deformation of the egg prior to the first cleavage. We shall now consider in a general way the events in each of the three intervals.

TABLE I

Interval	Event	Time after insemination
		minutes
	Insemination of the egg	0
	Breakdown of the germinal vesicle	15–20
A	Final incorporation of sperm	40–45
	First polar body formation	45–50
B	Second polar body formation	60–65
C	First cleavage	90–100

The interval designated above as 'A' is characterized by certain changes in the nucleus of the egg and by the gradual incorporation of the sperm. The germinal vesicle breaks down as indicated in the table and the contents of this cell organ approach the surface of the egg near the polar end. Toward the latter part of the interval the spindle of the first polocytic division forms from this material. In the meantime the cytoplasm of the egg itself displays an activity which appears to be just as characteristic. Immediately before and during the breakdown of the germinal vesicle, the egg goes through a series of slow labored pulsations which deform the egg, now in one direction, now in another. Some of these are quite extensive. The deformations appear at their greatest just before and during the actual disappearance of the membrane of the vesicle. They continue up to approximately 25 minutes after insemination, when the egg appears to enter a short 'rest period' during which they practically cease. The egg emerges from the 'rest period' after from 3 to 5 minutes and upon regaining the active state

continues changing its shape by pulsating without pause throughout the remainder of the period which ends at about the time of the disappearance of the sperm head within the egg. At no time are the movements as great following the 'rest period' as during the breakdown of the vesicle. There is some difference between the general appearance of the pulsations which appear prior to the 'rest period' and after. These will be considered below.

The second interval (interval 'B') covers the period between the complete separation of the first polar body and the initiation of the elevation of the second. During this period, as during the first, the activities of the zygote may be seen to center around the female nucleus, the sperm head, and the cytoplasm of the egg. The egg nucleus, now that of the secondary oöcyte, forms the second maturation spindle. The sperm head swells and becomes reoriented within the outer layers of the cytoplasm near the site of penetration. In addition, the interval is characterized by a succession of pulsations of the same sort as those noted during the first interval. The pulsations are probably even less extensive than those following the 'rest period' of interval 'A' though the difference is slight. There is no doubt, however, but that the interval represents a period of increased activity so far as egg movement is concerned.

Interval 'C' extends from the completion of second polar body formation to the beginning of the first cleavage. It is characterized within the nuclear elements of the zygote by the formation of the female pronucleus, the establishment of the male pronucleus, the migration of both to a central position in the egg with the development of the asters, and the breakdown of the united nuclei in the formation of the first cleavage spindle. The time required for this series of events varies somewhat as indicated above. In general the period is one of activity on the part of the egg. The gross form is altered constantly by pulsations though these are not as extensive as in interval 'A.' They do compare well with those of the second interval. When the whole interval is examined in detail, it is evident that there is not an immediate resumption of activity after the formation of the second polar body as after the formation of the first. Only after a considerable lag does one find the pulsations of the egg at all extensive. The peak is reached about halfway between the elimination of the polar body and the time of the appearance of the first cleavage plane. This is probably very significant, as will be shown below. Just prior to the extensive gross deformation of the egg which precedes the first cleavage, the presence of pulsations is masked by the elongation of the egg and the flattening of the poles which accompany the formation of the spindle and the

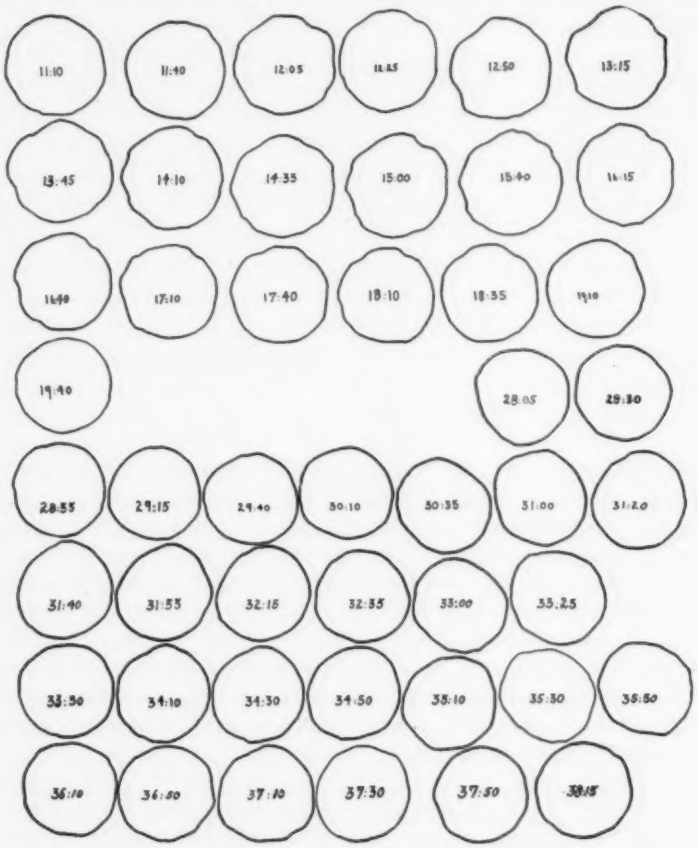
appearance of the furrow. The cessation of the pulsations prior to and during cleavage is not as difficult to understand as that occurring during the formation of the polar bodies, for the former involves the entire egg while the latter involves only the polar region immediately adjacent to the maturation spindles and hence but a relatively small portion of the entire surface.

In the above descriptions only the periods designated by the intervals have been considered. Nothing has been said about the period of sperm head incorporation, polar body formation, or cleavage save that the pulsations of the egg are interrupted. The interruption is not abrupt but gradual. As the egg nears each of these stages, the outer region becomes more regular as the extent of the movements decreases. It also appears more rigid than either before or after. It has much the same appearance during the 'lag period' which follows the isolation of the second polar body. At the time of the first cleavage, not only does the surface of the egg appear more regular but the entire egg becomes elongated in the equatorial plane in such a way as possibly to dominate or mask secondary activity. This is also true just before the appearance of the second cleavage plane.

The extent of the deformations which have been described in general above may be better appreciated upon examination of the text figure which represents tracings of a single egg during interval 'A.' It is a part of a complete record of the egg but only this period will be considered in detail inasmuch as two general types of activity are found during this period and inasmuch as the deformities at this time are of such magnitude that they are easily recognized by comparing the tracings. The period prior to and during the breakdown of the germinal vesicle is represented by nineteen tracings taken between 11 minutes and 10 seconds and 19 minutes and 40 seconds after the insemination of the batch of eggs to which this example belonged. Careful examination will show that the pulsations of the egg are of two sorts, one of which is quite extensive and results in general distortion of the sphere, and the other of which results in surface irregularities which appear more or less localized. After the 'rest period' which ended at about 27 minutes, the egg resumed its pulsations, which may be seen to be quite extensive. These pulsations are of the more general type with no evidence of the more localized irregularities. By 37 minutes and 50 seconds and 38 minutes and 15 seconds the egg is rounding out and appears rigid as the time for the inclusion of the sperm is neared. The deformations of the intervals described above as 'B' and 'C' are similar to those of the second portion of interval 'A,' but in this ex-

ample are not as extensive and are more difficult to follow in tracings. They are not included here.

The period between the completion of the first cleavage plane and the initiation of the formation of the second is similar to that of interval



Deformations of the egg during interval 'A.' For explanation see text.

'C.' Slow labored deformations occur which, however, are not as extensive as those noted in any of the intervals described above. They are retarded and finally cease as the blastomeres elongate prior to the second cleavage. In this connection two sets of observations may be of interest. In early experiments, the drop of sea water was not as well protected on the slides as in later ones. The evaporation of the

medium resulted in a marked hypertonicity of the solution. In such cases the eggs often did not successfully complete the first cleavage. Where no cleavage took place, the pulsations were somewhat decreased in extent even before the time of division in the controls. In addition, they ceased entirely during the cleavage period, only to reappear, though they were of lesser amplitude, after the cleavage was completed. They ceased a second time at approximately the time at which the second cleavage might have been expected. The fact that just after the cessation of the movements the eggs collapsed seems to indicate that they were approaching that stage, for it has been shown by several workers that permeability is increased at the time of cleavage. The general observations have been confirmed on eggs placed in slightly hypertonic sea water just before the first cleavage. None of the eggs were followed beyond this stage.

Discussion

Inasmuch as the above observations were made upon the relatively rigid egg of *Nereis*, it must be assumed that pulsations of this sort, found to occur in the eggs of some of the cœlenterates and described there as amœboid, are also characteristic of the eggs of many other invertebrates and probably of the eggs of some vertebrates as well. In some groups these are extensive; in others less so. In some they are evident only in the earliest developmental stages; in others they may be observed over a much longer interval. Those who are familiar with the moving picture films of fertilization and the early development of the eggs of several marine invertebrates taken by Professor Robert Chambers will recall the occurrence of movement there. The phenomenon must be much more general than might at first be thought.

In considering the portion of the egg cytoplasm responsible for the pulsations, account must be taken of both the cortical region and the deeper, more central endoplasmic mass. The localized irregularities in the surface which appear during the earlier part of interval 'A' would seem to be the result of activities in the cortical layer. It is more difficult to localize the centers responsible for the more extensive movements.

While the events described above must be significant in the development of the zygote, they apparently do not result in any immediately recognizable change in its intimate constitution. The continuous occurrence of the deformations of the egg (see below) indicates strongly that they are, to a large extent, independent of immediate nuclear control being initiated and accomplished within the cytoplasm during all phases of nuclear activity. Similar independence of nuclear and cytoplasmic

phenomena has been demonstrated in a number of places, more notably by Lillie (1902, 1906) and Wilson (1904). On the other hand, the importance of a certain minimal amount of chromosomal material to the completion of cleavage has been emphasized by Dalcq and Simon (1932).

Perhaps the most interesting relationship is that existing between the magnitude of the change in shape and the varying physical state of the egg protoplasm as it has been described for numerous other forms by Heilbrunn. This worker (1928) states that the egg of *Nereis* has a relatively high viscosity when shed into sea water. This decreases greatly with the breakdown of the germinal vesicle, temporarily to increase again three times. These increases Heilbrunn associates with the formation of the meiotic spindles and of the spindle of the first cleavage. There is no mention of a fourth increase immediately after the breakdown of the germinal vesicle during what has been called the 'rest period' above, but the description is given only for comparison with the egg of *Cumingia* which has been very carefully studied. Pantin (1924), working with the unripe eggs of *Nereis diversicolor*, obtained results on the effect of temperature on viscosity which differs slightly from those of Heilbrunn for *Arbacia* and *Cumingia*.[2] It is probable, however, that the egg of the heteronereis form of *Nereis* and that of *Cumingia* are comparable. If the graph made by Heilbrunn (1921) to express the relative viscosity of the *Cumingia* egg during the pre-cleavage period be inverted, the 0 minutes be taken as 10 minutes in *Nereis*, and the time of the maturation divisions and the first cleavage fitted, the curve represents the extent of the deformations very closely with one exception. That exception is in the 'rest period' described for *Nereis* during which the pulsations cease. It is probable that the pulsations which are evident during certain phases of the development of the egg are really characteristic of all stages but are masked at certain times by the gelation of the cytoplasm of the egg as a whole. It is of interest to note that even the lag after the formation of the second polar body may be explained by reference to Heilbrunn's diagram.

It was noted above that when the eggs were developing in a medium which was hypertonic, the activity was not as extensive as in normal sea water. Similar results were obtained by Pantin (1923), who has observed that the activity of the marine amœba (*Limax* type) was reduced in hypotonic and hypertonic media.

In conclusion, the results of the observations may be summarized

[2] Costello (1934), working in Heilbrunn's laboratory, has found that the effects of temperature on the viscosity of the egg of *Arbacia* follow closely those described by Pantin for *Nereis*.

briefly as follows. After insemination the egg of *Nereis* shows a series of deformations which are continuous and which decrease in extent up to the time of the second cleavage. These are masked periodically by more general changes in the physical state of the entire cytoplasm of the egg, as described by Heilbrunn, so that they appear to occur in waves. It is possible that these activities on the part of the cortex culminate in the formation of the cleavage planes but at the present time the evidence does not warrant such a statement.

LITERATURE CITED

COSTELLO, D. P., 1934. The Effects of Temperature on the Viscosity of Arbacia Egg Protoplasm. *Jour. Cell. and Comp. Physiol.*, **4**: 421.

DALCQ, A. M., AND S. SIMON, 1932. Contribution a l'analyse des fonctions nucléaires dans l'ontogénèse de la grenouille. II. Le rôle dynamique des chromosomes mis en évidence par lésion mécanique ou irradiation des gamètes. *Protoplasma*, **14**: 497.

HEILBRUNN, L. V., 1921. Protoplasmic Viscosity Changes during Mitosis. *Jour. Exper. Zool.*, **34**: 417.

HEILBRUNN, L. V., 1928. The Colloid Chemistry of Protoplasm. Berlin, 1928.

LILLIE, F. R., 1902. Differentiation without Cleavage in the Egg of the Annelid Chætopterus pergamentaceus. *Roux's Arch.*, **14**: 477.

LILLIE, F. R., 1906. Observations and Experiments Concerning the Elementary Phenomena of Embryonic Development in Chætopterus. *Jour. Exper. Zool.*, **3**: 153.

PANTIN, C. F. A., 1923. On the Physiology of Amœboid Movement, I. *Jour. Mar. Biol. Assn. Plymouth*, **13**: 24.

PANTIN, C. F. A., 1924. Temperature and the Viscosity of Protoplasm. *Jour. Mar. Biol. Assn. Plymouth*, **13**: 331.

SPEK, JOSEF, 1926. Über gesetzmässige substanzverteilungen bei der furchung des Ctenophoreneies und ihre Beziehungen zu den Determinationsproblemen. *Roux's Arch.*, **107**: 54.

WILSON, E. B., 1904. Experimental Studies on Germinal Localization. I. *Jour. Exper. Zool.*, **1**: 1.

AUTOTOMY IN THE ANOMURAN, PORCELLANA PLATYCHELES (PENNANT)

LEIGH HOADLEY

(*From the Department of Biology, Harvard University,*[1] *Cambridge*)

The autotomy of parts of the individual has been observed and studied during some stage of the life history of representatives of many of the phyla of the animal kingdom. Generally speaking, animals showing this phenomenon may be subdivided into two groups, those in which the autotomy is followed by a regeneration of the lost part, and those which do not replace the missing structures. Among the latter are to be found many animals such as the echinoderms and various parasites whose adult form differs greatly from that of the larva. The first group, while not restricted to the Arthropoda, is probably best represented by that group, many of which rid themselves readily of appendages by autotomy, autospasy, or autotilly [2] for a variety of reasons. For the most part, the numerous investigators who have studied autotomy have been interested primarily in the mechanics of the process itself or in the events of the subsequent regeneration. The present observations are concerned with the extent to which an animal will cast off its appendages in response to mild stimulation, and with some apparent correlations between the sex of the individual and the amount of autotomization. Attention was first called to this aspect of the phenomenon while numbers of the small anomuran, *Porcellana platycheles*, were being examined for developing eggs.

Material

Porcellana platycheles [3] is an anomuran closely related to *Galatheinea*. The carapace of the animals used in the experiments measured approximately 8 mm. in length and 7 mm. in breadth. *P. platycheles* is

[1] This investigation was aided by a grant from the Milton Fund. I wish here to express my gratitude to the College de France for the use of space in the Laboratoire Maretime at Concarneau and to the Director, M. Réné Legèndre, and to M. E. Fauré-Fremiét, who combined to make my stay in the laboratory a pleasant and profitable one.

[2] Definitions of the methods of self-mutilation and their occurrence in Crustacea have been treated very completely by Wood and Wood (1932).

[3] I wish to thank Dr. F. C. Chace for the identification of the anomuran. An illustration of this form may be found in the *Challenger Reports* for 1873–76, *Anomura* (J. R. Henderson), Plate XI, Fig. 7. *Porcellana platycheles* and its distribution are described by Milne Edwards in "Histoire Naturelle des Crustaces," T. II, p. 255, Paris 1837.

a squat form with chilipeds which are broad and flattened and are drawn close to the head parts as it clings to the rocks on the lower side of which it is found. The three anterior walking legs are well developed while the fourth is slender and inflexed. The pleon is slender and is utilized in locomotion. Females bearing eggs are quite thick and have some difficulty in holding themselves to the rocks when disturbed.

The animals are very common on the coast of France, especially in the region south of the Manche along the shores of the Bay of Biscay. All of the animals used in the present report were collected on several small rocky islands which are located in the entrance to the outer harbor of Concarneau in Finistére. Stones and rocks in the region between the high and low tide levels were turned over and the animals found clinging closely to the roughened lower sides. They were touched and immediately dropped into a bucket in which they were transferred to the laboratory. The collection was made the last week of June, 1930, when all of the females collected save one were bearing eggs.

The appendages of *Porcellana platycheles* are autotomized upon the slightest provocation. Repeated attempts were made to preserve the animals intact for further study only to find that as soon as the animal was put into fixing fluid most of the legs were cast off. Of those which were collected and brought to the laboratory, some had lost appendages in transit. Many of the animals, however, retained all of the legs up to the time of the experiment.

Methods

The experiments were of two kinds, both of which were very simple. Isolated animals with all of the appendages intact or with but one of the chilipeds or walking legs missing were placed in dishes in a small amount of fresh sea water and allowed to remain unmolested until they began to move freely about the container. Without moving the dish, one of the appendages was gently seized with a pair of blunt forceps and the occurrence or non-occurrence of autotomy recorded. The process was repeated with another leg and continued until each one of the appendages had been tested once. Only the chiliped and the three anterior walking legs of each side were tested inasmuch as the fourth pair is short and slender and not easily grasped without touching the owner. The chiliped of the right side was designated as 1, and the walking legs as 2, 3, and 4; those of the left side correspondingly as *A*, *B*, *C*, and *D*. Each experiment therefore involved eight separate tests.

The above experiment was varied slightly with ten of the animals. Legs were stimulated one after the other, continuing until all had been dropped. In some cases each leg was stimulated as above while in three

there were repetitions before all of the legs had been tested. It was soon found that the last remaining appendages did not autotomize after

TABLE I

Autotomy by males. The letters and figures in Roman type indicate appendages autotomized; those in italics represent appendages retained. The two cases omitted in the averages are considered in the text.

Animal	Remarks	First 4 trials					Second 4 trials					Total 8 trials
		1	2	3	4	Total	1	2	3	4	Total	
I		C	A	B	D	4	2	*4*	*3*	*1*	1	5
III		A	2	C	4	4	*D*	1	*3*	*B*	1	5
VII		1	D	3	A	4	B	4	2	*C*	3	7
IX	Right antenna lost	D	2	A	3	4	C	4	*B*	1	3	7
X	Left antenna removed	D	4	B	2	4	A	1	C	*3*	3	7
XI	D missing	1	A	2	B	4	3	*C*	*4*		1	5 (6)
XII		1	A	2	B	4	3	C	*4*	*D*	2	6
XIII		4	D	3	C	4	2	*B*	*1*	*A*	1	5
XIV		4	D	3	C	4	2	*B*	*1*	*A*	1	5
XV		1	A	2	B	4	3	*C*	*4*	*D*	1	5
XVII		4	D	3	C	4	2	*B*	1	*A*	2	6
XVIII	A missing	*1*	*2*	*B*	*3*	0	*C*	*4*	*D*		0	0 (1)
XIX	A, 1, B missing —Large animal—	*2*	*3*	C	4	2	D				1	3 (6)
XX		1	A	2	B	4	3	*C*	*4*	*D*	1	5
XXI		4	D	3	C	4	*2*	*B*	*1*	*A*	0	4
XXII		2	B	3	C	4	1	A	*4*	*D*	2	6
Average (XVIII and XIX omitted)						4					1.57+	5.57+

numerous trials, so that the member was injured either by cutting with a pair of scissors or by crushing with forceps. Even then the leg was sometimes held for a time before being freed. The seven cases which

were treated in the same way as those in the experiments described in the preceding paragraph will be considered in both connections.

Experiments

We will consider first the results of the experiments in which each leg was stimulated but once, a total of eight successive trials. The data are presented in tabular form in Tables I and II. After a consideration of the more general aspects of the results, each of the cases which is distinguished by some qualification in the column headed "Remarks" will receive separate attention.

It is apparent after but a brief examination of the results of the experiments as they are presented in Tables I and II that there is a

Table II

Autotomy by females. The letters and figures are used as in Table II. Case VIII is considered in detail in the text.

Animal	Remarks	First 4 trials					Second 4 trials					Total 8 trials
		1	2	3	4	Total	1	2	3	4	Total	
VI		*1*	*A*	*4*	*2*	0	C	*D*	3	*B*	2	2
VIII	No eggs	B	4	D	2	4	*A*	1	*C*	*3*	1	5
XVI		*4*	D	3	C	3	*2*	*B*	*1*	*A*	0	3
XXIII		2	B	*3*	*C*	2	*1*	*A*	*4*	*D*	0	2
XXIV	Laying eggs	2	*B*	*3*	*C*	1	*1*	*A*	*4*	*D*	0	1
Average (VIII omitted)						1.5					0.5	2

great deal of consistency in the behavior of the animals. Leaving for special consideration Animals XVIII and XIX, it will be seen that each of the males tested cast off an appendage at each of the first four trials. During the second four the number of the legs cast varied from 0 in XXI to 3 in VII, IX, and X. It is also of interest to note that the order in which the appendages were tested appears to have little to do with the final result. In some cases the initial trials were made with the chilipeds, in others with the fourth pair, and in still others with one of the intermediate pairs. The average of the number of legs cast by the males on the first four trials was 4, and on the second four, 1.57 +. While some of the animals will cast as many as seven legs in the eight trials, the average is between five and six.

Unfortunately the number of females on which records were made is relatively small. This in itself was a result of a difference in the behavior of the two sexes. As may be seen from Table II, the females do not autotomize as readily as do the males, at least while they are bearing eggs. Inasmuch as many of those with developing eggs were preserved before the experiments were begun, fewer remained for the actual tests. Those preserved did not drop more than one or two of the appendages until placed in the fixing fluid. Even then they generally retained some of their members. Disregarding for the moment the results obtained with Animal VIII, the only female examined which did not bear eggs, it will be seen that the females as a group cast from 0 to 3 legs on the first four trials and from 0 to 2 on the second four. The average for the first four is 1.5 and for the second four is 0.50. The average for the eight is 2. Only one of the females autotomized any appendage in the second period of the experiment and there the number cast was two. These averages, which are to be found in Table II, should be compared with those for the males in Table I. The difference in the behavior of animals of opposite sexes must be significant.

We will now consider those cases which have been qualified in the table.

Nothing need be said of Animal XI save that the third walking leg of the left side was missing when the experiment was begun. The total number of legs cast before the animal ceased to react was therefore 6 instead of 5, as would appear from the record. Number XIX lacked 3 of its legs but was tested because it was an unusually large animal. During the trials three more of the legs were dropped, which brings the total in that case up to 6 in place of the 3 which appear in the table. Inasmuch as so many of the legs were missing when the experiment was begun, it was considered legitimate to omit this case from the averages made and presented above. Female XXIV was laying eggs at the time the experiment was performed. This animal autotomized but one leg and that on the first trial.

One male (IX) was tested from which one of the second antennæ was missing. As has been mentioned above, the flagellum of the antenna is very long and hence is easily injured. The animal cast 7 of its legs in eight trials, failing on the seventh. The next animal experimented upon was also a male, so that the left second antenna was removed by scissors before the legs were touched. That animal (*X*) also dropped 7 of its legs, failing only on the eighth trial. Inasmuch as Animal VII also dropped 7 of its legs and a number dropped 6, no other experiments of this sort were made.

Of all of the females collected and examined only one (VIII) had

no eggs attached. Not only did this animal bear no eggs, but its behavior in autotomy was similar to that of the males. It is possible that an error was made in sexing the animal, which, unfortunately, was not preserved, but that seems highly improbable. It is possible that there was some functional disorder present which resulted in the absence of the ova. It is also possible that the animal had already laid its eggs and that all of the young were hatched prior to the time of collection. Inasmuch as the rest of the females bore eggs which were far enough along in their development to show eye spots and yet not old enough to be hatched, this does not seem probable. The animal was not included in computing the averages.

Male XVII is another case in which an animal showed an autotomy reflex which was characteristic of the opposite sex. The left chiliped was missing when the experiment was begun. No appendages were cast during the seven trials which were made on the remaining members. The behavior, therefore, was characterized by more inhibition to autotomization than is generally found in the female. It is probable that the absence of the autotomy reflex should be explained on some of the grounds to be discussed below. If the animal had recently molted, for example, autotomy would not be expected. In that case, however, the absence of the left chiliped must be attributed to autospasy or combat, and not to true autotomy. Inasmuch as the reactions of the animal were so evidently atypical, it is not considered in connection with the averages above.

The second type of experiment involved the successive stimulation of appendages, one after another, until all or all but one had been autotomized. So far as possible, the data are summarized in Table III. Three sorts of information will be found embodied in the figures. They indicate the number of trials made on any specific leg and the number of the trial (in the total number) at which the leg autotomized. They also indicate whether the leg was dropped in response to the mild stimulation employed throughout in the experiment above or in response to injury either by cutting or by crushing. Occasionally a leg is retained even after injury and later released, apparently in response to mild stimulation (I, II). On the other hand, in three of the cases (VI, IX, X) injured members were held to the end of the experiment. Seven of the cases reported here are also to be found reported in a slightly different way among the experiments of Tables I and II.

It is immediately apparent on examination of the table that the females tend to retain the legs longer than do the males. This is evident not only in the larger number of attempts required to cause all of the legs to be released but also in the number of legs which have to be injured before they are autotomized. It is also evident, both in the

TABLE III

Autotomy on repeated stimulation. In the columns headed "Trial on which the leg was cast," the subscripts indicate the number of trials with that leg. Where no subscript is present it is understood that the leg was cast on the first trial. In the column headed "Injury," the subscripts indicate the number of the trials at which that particular leg was injured.

Animal	Remarks	Trial on which the leg was cast									Total trials	Sex
		1	2	3	4	A	B	C	D	Injury		
I		11_2	5	18_5	17_6	2	3	1	4	3_5 4_5	18	♂
II		28_4	17_2	25_5	33_8	16_4	18_3	36_9	6	A_4 C_7 1_4	36	♀
III		6	2	28_{10}	4	1	25_8	3	17_5	B_8 D_5	28	♂
IV	Both antennæ removed	26_8	6	10	8_2	25_{11}	7	9	5	A_{11} 1_8	26	♂
V	Twelve trials	—	4	—	5	—	3	—	—	A dropped in Bouin's	—	♀
VI	B eight trials but not cast	22_4	18_3	7	6_2	16_4	—	5	17_2	A_4 B_7 D_2 1_4	25	♀
VII		1	7	3	6	4	5	11_4	2	C_4	11	♂
VIII		1	4	16_4	2	17_4	1	15_4	3	A_4 C_4 3_4	17	♀
IX	B eight trials but not cast	8	2	4	6	3	—	5	1	B_5 B_7	15	♂
X	3 eight trials but not cast	6	4	—	2	5	3	7	1	3_6	15	♂

males and the females, that a resistance to autotomy is built up as repeated trials are made and more and more of the legs are cast. Two males (IX, X) and one female (VI) failed to autotomize the last remaining leg even on repeated stimulation after injury. As in the data of the previous experiment, the record of animal VIII shows its behavior to be much more like that of a male than a female. Unfortunately, animal XVIII was not employed in this series.

DISCUSSION

The general question of autotomy in the decapod Crustacea has been discussed in considerable detail in a recent report by Wood and Wood (1932). Those desiring an account of the earlier work on this sub-

ject should consult their paper. The observations of these investigators cover a wide variety of forms found both on these shores and at Roscoff. Unfortunately they were unable to obtain *Porcellana* but they state concerning *Galathea squamifera*, a closely related anomuran, that it "autotomizes on less stimulation than any other form used." While they were interested primarily in the mechanics of autotomy, a number of their observations are of importance to the present discussion.

Porcellana casts its appendages by the method described by Wood and Wood as true autotomy. They distinguish between three types of self-mutilation, all of which are dependent upon the physiological state of the animal for their realization. The following observations are mentioned specifically here as they may have some bearing on the results obtained above. Animals kept in the laboratory for some length of time, even though apparently in good condition, may fail to show the typical autotomy reflex (*Cambarus* var. sp.). Loss of blood may so weaken an animal that in spite of a vigorous pinching response by the chilipeds, no typical autotomy can be induced. Experiments on *Carcinides moenas*, the green crab, have shown that newly molted animals are not able to autotomize their appendages. *Carcinides* infected with *Sacculina* autotomized as readily as did uninfected animals. No sex differences in autotomy were observed.

Turning now to the information obtained in the experiments reported above, it is quite apparent from Table I that animals with all of the appendages present autotomize much more readily than do animals which have already cast some of their legs. The act of autotomy in itself appears, therefore, to have a very definite influence on the reflex. There are two possible explanations for this fact which immediately come to mind. The animal may be in a poorer condition after the shedding of some of its legs so that it resembles in its reactions individuals which have been bled or have been held in the laboratory. On the other hand, the autotomy itself may alter the typical reaction of the animal by freeing some substance or substances which act as inhibitors to the process. This possibility should be tested. It is of interest to note that while the first legs to be cast are released after mild stimulation, extensive injury is often insufficient to cause the last legs to be dropped. The inhibitory influence must be a potent one.

The order in which the appendages are stimulated and dropped has little or no effect upon the total number which will be cast by an individual. When the experiment is begun with the anterior appendages, the two posterior walking legs will usually remain, while if the posterior walking legs are the first to be tested, the two chilipeds are retained. One complete experiment of the first type required much less

than five minutes, so that the factor or factors responsible for the change in the reflex must act very quickly. Inasmuch as the legs are freed by true autotomy it may be assumed that the act is accompanied by very little loss of blood. The evidence does not favor a control by inhibition due to the lowered "vitality" of the animal.

A single test was made with an animal which had previously lost more than one of its appendages. This was a large male with both of the chilipeds and the first walking leg of the left side missing. Each of the five remaining members was tested once with the result that three of them were autotomized. The total number of legs cast by the animal was therefore six, which compares very favorably with the total number dropped in eight trials by intact animals. If the cause for the retention of the last legs is to be found in the production of some substance which modifies the reactions of the individual, either this animal must have lost the three members just before the beginning of the experiment or the effects of the autotomy of the limb must persist for some time. The results obtained with those animals from which one (IX and X) or both (IV) of the second antennæ were missing when the experiment was begun have a distinct bearing on this question. Mutilation of one antenna apparently has no effect on the behavior in autotomy though mutilation of two may have such an effect. Were the change in the reflex the result of loss of blood, it might be expected that injury to the antennæ would be as potent as injury to a leg in this connection. The evidence in the experiments does not confirm this hypothesis. The effect appears to be intimately associated with the loss of the legs themselves.

Both of the tables prepared in connection with the experiments contain definite evidence that the behavior of animals of the two sexes differs in respect to autotomy.[4] Males cast more of the legs than do females carrying eggs and in addition they cast the legs more readily than the females. It is unfortunate that the number of the females examined was so small but, as stated above, the primary interest in the females carrying eggs led to the preservation of many before the experiments were commenced. There is such a high degree of consistency in the results obtained that the relatively small number may be taken as representative of the group. The difference in the behavior of the two sexes might be attributed to a structural difference which would make the reflex mechanically difficult. Wood and Wood (loc. cit.) have stated that animals with heavy exoskeletons do not easily accomplish true autotomy. No difference in the nature of the carapaces was

[4] M. R. Legèndre has informed me that he has noted similar sexual differences in *Porcellana platycheles*.

noted here. The presence of eggs produces changes in the gross relations between the parts of the body which might be thought to be significant. Wood and Wood have shown, however, that *Carcinides* infected with *Sacculina* can autotomize its appendages. The relationships in that case would be similar to those in animals bearing eggs. The parasite is found on the males but as the same workers have shown, there is no difference in the mechanics of the process in the two sexes. Moreover, the very fact that animals of both sexes will drop some of their legs by true autotomy shows that the reflex may be accomplished by both.

The difference in the behavior of the males and the females carrying eggs is more satisfactorily explained on the assumption that there is some fundamental difference in their functional status which modifies the reflex. It is well known that the sexes differ as to their metabolism. Moreover, in some Crustacea, animals of opposite sexes have dissimilar pigmentation during the breeding season (shrimps).[5] In the Insecta sex differences in behavior are found in the termites and the ants where the female autotomizes the wings at the conclusion of the nuptial flight. Similarly, some of the parasitic flies lose their wings when they reach the host on which they take up their permanent abode. The alteration or differentiation of the behavior pattern according to the mode of life or the sex of the individual is a very common occurrence. The evidence in the present case points to a similar fundamental difference in the reactions of the two sexes in *Porcellana*. It would be very interesting to know the behavior of females at other seasons of the year. Some indication might be inferred from female VIII, but this is an isolated case and should not be taken as representative of the group without further experimentation.

The discussion would not be complete without some brief reference to the data obtained in relation to adaptation. Autotomy itself has been considered from this standpoint so often that nothing further need be said here. The behavior of the females in relation to the males is of interest for during the breeding season the female is less active than the male and hence less liable to injury. Moreover, the gravid female is continually dependent upon her appendages both in the care of the eggs and in defense, while the mode of life of the more active male changes but little. Fortunately the male, which is more subject to injury, autotomizes more readily than the female, which makes constant use of its legs.

LITERATURE CITED

Wood, F. D., and H. E. Wood, II, 1932. Autotomy in Decapod Crustacea. *Jour. Exper. Zoöl.*, **62**: 1.

[5] I am indebted to Dr. Frank A. Brown for this information.

EXPERIMENTAL PROCEDURES IN A STUDY OF THE LOCATION AND CONCENTRATION WITHIN THE HOST CELL OF THE VIRUS OF TOBACCO MOSAIC

L. G. LIVINGSTON AND B. M. DUGGAR[1]

(From the Botanical Laboratory, University of Wisconsin)

It has long been felt by many investigators that the active agent in any typical mosaic disease of plants may be intimately associated with the protoplasm of the affected host. This view is no doubt due in part to the mass of evidence indicating rapid multiplication and migration of the virus in the living tissues of the host, and the lack of convincing evidence that such virus agents may be propagated *in vitro*. It is quite conceivable that the virus agents of many animal diseases reach body fluids and secretions, and ultimately find ready means of transfer to other host individuals. Since in plants there are no such general fluids, with the exception of that of the "transpiration stream," it seemed particularly desirable and important to direct efforts toward the determination of the location and concentration of the virus agent in the various parts of the living cell, including, of course, the various protoplasmic components and the vacuole. It appears that no such study has previously been attempted. The "typical" mosaic of tobacco offered many advantages and was selected as the main object for the preliminary studies here reported.

Methods and Materials

Micro-methods

A Chamber's micro-manipulation apparatus, consisting of two manipulation units and a Leuer syringe attachment for extraction, was used in this work. The operations were carried out under a Spencer microscope equipped with a mechanical stage, using a 4 mm. objective and a 6 × ocular, giving an approximate magnification of 260 ×.

The micro-pipettes were made from hand-drawn capillary Pyrex tubing with an approximate inside diameter of 0.5 mm. and a wall thickness of approximately 0.1 mm. The tips of the pipettes were drawn out to a fine point with a fairly abrupt taper, ending in a tip with an opening of from 0.5 μ to 1. μ in diameter. It was found necessary to have

[1] This work was made possible, in part, through a grant from the Research Committee, Graduate School, University of Wisconsin

the taper fairly abrupt and uniform to the very tip in order to prevent clogging and to facilitate withdrawal of the cell contents. Inasmuch as the type of pipette suited for this work was very difficult to make by hand, a mechanical device was designed which produced very uniform pipettes of the type required. This device, which embodies certain modifications of the principle utilized in an earlier machine designed by Du Bois,[2] will be described in a subsequent paper (Livingston, 1934, in press). The tips of the pipettes were bent upward at an angle of 15 to 20°, sealed into the special adapters with DeKhotinsky cement, and attached to the tube leading from the syringe, which was mounted on one of the manipulator units. In those cases in which a larger opening in the tip of the pipette was desired for some of the work, the tip was broken off under the microscope with a needle attached to the other manipulator, leaving a jagged opening with a diameter of 2 or 3 μ.

After considerable experimentation, 20 per cent gelatin was used as a mounting medium. This was used because of the following advantages it possessed over the usual liquid mounting media: (1) It holds the tissue firmly in place, so that it is easy to puncture and to enter the cell to be operated upon at any point desired, this firmness being unattainable in liquid media with the tissue used in these investigations. (2) Since the gelatin is solid, there is no danger of the mounting medium entering the pipette and diluting the extracted material. (3) The low solidification point of the gelatin, which is slightly above room temperature, makes it possible to apply it in a liquid state without danger of injuring the tissue by excess heat.

Because the hair cells of the tobacco plant are readily accessible and are sufficiently large to be easily operated upon, and are known to contain a high concentration of the agency causing the mosaic disease, these cells were used in this study. Freshly diseased shoots from young, rapidly growing plants were used, and thin cross-sections were cut with a sharp razor from the base of the midrib of a diseased leaf or from the first internode of the stem above the lowest leaf showing definite mosaic symptoms. These sections were mounted on a 24 x 30 mm. cover slip in a drop of gelatin as described above. Several sections were cut at one time, and the mounted material was kept in a closed Petri dish on moist filter paper until operated upon. Except where specified below, no material was used in these studies which had been mounted more than 6 hours. Over this period of time, the cells remained fully turgid and exhibited active streaming.

For operation upon the cells, the cover-slip with the mounted material was inverted over a moist-chamber and was held in place by a coating of Lubriseal. It was found that cooling the tissue markedly

[2] *Science* (N. S.), **73**: 344–345, 1931.

increased the viscosity of the protoplasm, which greatly facilitated certain operations. When this was desired, the material was thoroughly chilled on ice directly before inverting the cover-slip over the moist-chamber, and the moist-chamber was kept surrounded by a stream of brine from an ice-salt mixture throughout the operations.

As it was impossible to measure directly the quantity of material withdrawn, the portion of the pipette which was filled in the operation was carefully measured with a micrometer ocular, and the cubical contents computed. For each run, from 0.15 to 1 cu. mm. of material was collected, and this was diluted with distilled water to the concentration desired for inoculation. As it was impossible to expel all of the material from the pipettes, and the possibilities for adsorption were very great with such minute quantities, the tips of the pipettes were broken off and ground in a micro-mortar in the water used for dilution in order to free as much material as possible.

All inoculations were carried out by a standardized needle-prick and scratch method, using healthy potted plants grown in the greenhouse. As a control, thoroughly macerated hair cells, from the same diseased plant used for the micro-manipulation work were diluted to a concentration of 1/1,000 and inoculated in the same manner.

Macro-methods

It seemed probable that an application of Chibnall's [3] technique for securing separately samples of vacuolar sap and of protoplasmic extract might be made useful in the present study, and although recognizing the limitations of such macro-methods, this procedure was modified to suit our requirements.

The tobacco shoots infected with the virus of typical tobacco mosaic were grossly washed of any adhering dust or soil, dried of surface water under an electric fan, dipped and held in ether 3 minutes, and then immediately placed in an hydraulic press and subjected to a pressure of about 2,000 lbs. per square inch of plunger area. It is recognized that, while some of the cells are ruptured by this process and doubtless some unfiltered sap is thus obtained, it is also true that some sap is obtained which is filtered through protoplasm and cell wall under pressure. In the experiment referred to a volume of 30 cc. of juice, designated vacuolar sap, was obtained. This was later diluted (sample A) for the inoculations, as indicated in Table I. The remaining tissue residue was ground finely twice in a food grinder, 70 gm. were recovered, and this was diluted with 250 cc. distilled water, in order better to extract the virus. The residue was then pressed at 4500 lbs., and the "infective" juice collected was brought up to a volume that would yield a concentra-

[3] *Jour. Biol. Chem.*, **55**: 333–342, 1923.

tion of approximately 1/10. From a part of this juice (sample B) the other required dilutions were prepared. Another part of this 1/10 juice (sample C) was also treated with diatomaceous earth [4] and then diluted for inoculation as in the case of sample D.

Shoots of young tobacco plants infected with the virus of yellow mosaic were treated in precisely the same manner, as in the case of the typical mosaic, for the preparation of corresponding samples of this virus. All inoculations were carried out by a standardized needle-prick and scratch method, using potted plants grown in the greenhouse.

Results and Discussion

Some Physical Properties of the Cell Inclusions and the Relation of These Inclusions to the Concentration of the Virus Agent in the Cell

Although numerous cytological investigations have been made on the cell inclusions accompanying various virus diseases, micro-manipulative technique offers not only a new approach to the problem of sampling, but also in the present studies has made possible certain interesting observations on the physical properties of these cell inclusions.

There are two recognized types of intracellular bodies or inclusions which normally accompany the typical mosaic disease of tobacco, viz., the vacuolate type, sometimes designated as amœboid or X bodies, and the "crystalline" type or "striated" bodies. Both types are usually definitely associated with the cytoplasm of the cell. In certain of the hair cells, both types may be found; in others, they may occur separately. In the material used in the present studies, the "crystalline" bodies appeared to be much more numerous than the vacuolate type, and if the vacuolate type were present, they were usually accompanied by one or more of the crystalline type. As the "crystalline" bodies are often profoundly altered in appearance by the action of cytological fixatives, they are frequently overlooked in studies made on fixed and stained material.

The presence of either type of inclusion in the cell seems to bring about a rather marked decrease in the viscosity of the cytoplasm. It was noted that the cytoplasm of hair cells from a diseased plant which had no inclusions present, and which appeared to be normal in every respect, was sufficiently viscous so that on cooling to slightly above 0° C. it would not readily enter a pipette with an opening in the tip of slightly more than 1 μ in diameter. This was particularly true of the outer region, or the so-called ectoplasm. In contrast, the cytoplasm of cells with abundant inclusions would readily enter a pipette with a

[4] Duggar, B. M., *Proc. Soc. Exper. Biol. and Med.*, **30**: 1104–1109, 1933.

similar opening, and there seemed to be no noticeable differences in the viscosity of the various regions of the cytoplasm, regardless of cooling or of any other treatment resorted to. The phenomenon of semi-liquefaction of the cytoplasm would perhaps be expected in cells in an abnormal or pathological condition, such as that attending the presence of the virus agent and the formation of the inclusions.

The vacuolate inclusions appear to be surrounded by a more or less definite membrane or surface film which, for the time at least, maintains them in a definite form and preserves their identity. Ordinarily they will withstand considerable disturbance in the cytoplasm in their immediate vicinity, but if touched by a micro-needle or by the tip of a micropipette they break down immediately into a kind of granular mass, which readily enters a pipette with an opening of 1 μ in diameter. This observation seems significant.

The so-called crystalline bodies normally appear as flat, irregular, plate-like crystals, which, when seen on edge, often show a striated or palisade-like structure. Although in the living cell they often appear to be definitely crystalline in nature, they break down into a granular mass immediately on being touched or disturbed by the tip of a micropipette, and they readily enter the opening of the smallest pipette used. This behavior indicates that they are not truly crystalline.

Inasmuch as certain investigators have hypothecated that the inclusion bodies are actually concentrates, or at least an indication of high concentrations, of the virus within the cells in which they occur, it was deemed desirable to investigate the comparative concentration of the virus agency in hair cells from diseased plants which possessed abundant inclusions, in contrast with those cells, either from the same plant or from comparable plants, which possessed no visible inclusions of any kind. Accordingly, a young plant which had been inoculated 15 days previously, and which had shown definite mosaic symptoms for approximately one week, was selected, and sections were cut from the stem above the lowest leaf showing definite symptoms. These sections possessed abundant inclusions (both crystalline and vacuolate types) in the hairs in certain regions, while on other regions of the same section no inclusions were present (the former may correspond to the paler areas of leaves, the latter possibly to the grecner areas). The sections were mounted as previously described, and were operated upon at ordinary room temperature, using pipettes with an opening of 2–5 μ in diameter. Under these conditions it was possible to extract the entire cell contents, and material was collected from hair cells with abundant inclusions, as well as from hair cells on the same plant which possessed no visible inclusions. As a control, an inoculum was prepared from hair cells

clipped from the same diseased plant. All inoculations were made at a dilution of 1/1,000 on young, rapidly growing plants, using 10 plants for each series.

As may be noted on reference to Table I, *A*, the material from cells possessing abundant inclusions induced 80 per cent disease at the dilution used, while the material with no inclusions induced no disease. The controls were all diseased, as is usually the case in experiments carried out at a dilution of 1/1,000 with the technique used in these experiments. The slight diminution of the incident disease in the material collected from cells with abundant inclusions over the control is ascribed to adsorption and other difficulties involved in the micromanipulation technique.

TABLE I

Indications of the relation of inclusion bodies to the concentration of the virus agency within the cell.

	Material collected	Dilution for inoculation	No. plants inoculated	No. plants diseased	Percentage diseased
	mg.				*per cent*
A.					
Cell content (inclusions present).	0.249	1/1,000	10	8	80
Cell content (inclusions absent).	0.303	1/1,000	10	0	0
Control (macerated hairs).....	3.0	1/1,000	10	10	100
B.					
Cell content (inclusions present).	0.577	1/100	3	3	100
		1/1,000	4	4	100
Cell content (inclusions absent).	1.064	1/100	5	1	20
		1/1,000	10	0	0
Control (macerated hairs).....	2.0	1/1,000	10	10	100

For the purpose of further checking the results indicated in the above experiment, the test was repeated, collecting sufficient material to inoculate at a dilution of 1/100 as well as at 1/1,000. Diseased material from a later series, but of an age and condition similar to that used in the experiment described above, was used in this series. All sections employed possessed hairs with and without inclusions. However, as it was impossible to collect sufficient sap in one day's time to complete the experiment, different diseased plants were used as sources of virus for the various parts of this series, although the diseased material was from the same lot and in every way comparable. From the cells with inclusions present, 0.577 mg. of the cell contents was collected, an amount which, on dilution, was sufficient to inoculate 3 plants at a dilution of 1/100 and 4 plants at 1/1,000. As indicated in Table I, *B*, all plants

inoculated developed the disease. From the cells with inclusions absent, 1.064 mg. was collected, which, on dilution, was sufficient to inoculate 5 plants at a dilution of 1/100 and 10 plants at 1/1,000. Of these, one plant inoculated at 1/100 developed the disease, while the 1/1,000 series was entirely disease-free. The control, which consisted of hair cells collected from the same plant used for the material without inclusions, but which possessed abundant inclusions in various areas, induced 100 per cent disease.

The results of these experiments might indicate either that the inclusions are actual concentrates of the virus agency, or, as seems more reasonable, that they represent products accompanying the development of the agency in high concentrations. Inasmuch as these structures are intimately associated with the protoplasm of the cell, the suggestion may be offered that the protoplasm itself is the place of origin and development of the agency causing the mosaic disease of tobacco.

Preliminary Indications on the Location and Concentration of the Virus Agent within the Cell

Indications from Macro-methods.—As previously indicated, a modification of the Chibnall technique was used in these studies which were made on both typical tobacco mosaic and on the yellow mosaic of tobacco.

The results shown in Table II seem clearly to indicate that the sap pressed from the tissues with as little disintegration of the protoplasm as is possible with this technique contained the virus only in relatively low concentration. It should be remembered that ordinarily the incidence of disease at a dilution of 1/1,000, with the inoculation technique employed, averages around 90–100 per cent. On the contrary, the virus is found in the residue at full strength. Admitting the filtration possibilities of both protoplasm and cell walls, the results are nevertheless suggestive, and it was the more important to seek evidence from micro-manipulation (the micro-pipette method).

Indications from Micro-methods.—In these studies an attempt was made to remove the cell sap from diseased cells in as pure a state as possible and to contrast the infectivity of the cell sap with that of the entire cell contents from similar cells. In this way it was hoped to determine whether the virus agency was localized in the protoplasm or if it was present in high concentrations throughout the cell.

It must be recognized at the outset that any dissection or disturbance within the cell may bring about profound changes in its organization and condition, resulting in disturbances in adsorption and similar phenomena which might tend to seriously alter the results obtained. However, by

exercising care in the operation, and by working as rapidly as possible, these tendencies were no doubt greatly minimized, and while the results may not be strictly quantitative, they at least will serve as indications of the relative concentration of the virus agency within the various regions of the cell.

For the removal of the cell sap, only freshly mounted, thoroughly chilled hair cells from plants affected with "typical" tobacco mosaic were used. It was found, as indicated above, that it was important that the material used be in good physiological condition in order to effect fairly accurate fractionation. Accordingly, shoots from plants which were just beginning to show definite mosaic symptoms, having been

TABLE II

Indications from macro-methods as to the location of the virus agency within hair cells of tobacco plants affected with "mosaics."

Sample	Dilution for inoculation	Typical mosaic diseased	Yellow mosaic diseased
		per cent	*per cent*
Vacuolar sap, natural juice	1/1	100	100
	1/10	80	20
	1/100	50	0
	1/1,000	0	0
	1/10,000	0	0
Extract from tissue residue, natural juice	1/100	100	100
	1/1,000	90	90
	1/10,000	80	70
Extract from tissue residue, celite treated juice	1/100	100	100
	1/1,000	70	100
	1/10,000	40	40

inoculated ten days previously, were used in this experiment. The sections were cut from the first internode above the lowest leaf showing definite mosaic symptoms and were mounted as previously described. Approximately one-third of the hair cells possessed inclusions, chiefly of the crystalline type, although the cytoplasm of these cells still possessed sufficient viscosity to allow for fairly accurate fractionation of the cell sap versus cytoplasm if the material was kept at the proper temperature. The openings in the tips of the pipettes used did not exceed 2 μ, since openings of a larger size would allow a certain amount of cytoplasm to enter, regardless of other precautions. The pipettes were quickly thrust into the center of the cell, the cell sap withdrawn chiefly

by the capillary attraction of the pipette, supplemented occasionally by very mild suction applied to the syringe, and then quickly withdrawn. If any appreciable amount of cytoplasm entered the pipette under these conditions, it would be readily detected by the difference in refractive index and the behavior in the pipette, and the entire pipette would be discarded. Several pipettes were used in the extraction of the material, because the pipettes quickly lost their capillary action as the tips became filled, and often a small bit of cytoplasm would adhere to the tip and clog the opening.

The entire cell contents were removed from cells of similar plants with essentially the same technique described in the previous section, that is, operation on the cells at room temperature, and using pipettes with a larger opening. Hair cells were clipped from the plant used in the removal of the cell sap, macerated, and diluted 1/1,000 as a control.

TABLE III

Indications from micro-methods as to the relative concentration of the virus agency in the cell sap and in the cytoplasm of hair cells from diseased tobacco plants

	Material collected	Dilution for inoculation	No. plants inoculated	No. plants diseased	Percentage diseased
	mg.				*per cent*
Cell sap	0.577	1/1,000	10	2	20
Cell contents	0.581	1/1,000	10	8	80
Control	2.5	1/1,000	10	10	100

The results of a typical experiment (see Table III) indicate that the main concentration of the virus agency is in the protoplasm of the cell. The degree of infection resulting from the cell sap inoculations (20 per cent disease) may be due to the unobserved entrance of a small amount of cytoplasm or to the disturbance of the cytoplasm by the injury to the cell, resulting in the freeing of the virus agency into the cell sap. However, final conclusions on this matter must be withheld until it is possible to make a more thorough study than has been possible to date.

SUMMARY

In this paper experimental evidence is offered which strengthens the view that the virus of typical tobacco mosaic occurs primarily, if not solely, in the protoplasmic components of the cell, rather than in the vacuole. From the observations made it would seem clear that the occurrence of intracellular bodies, in hair cells at least, is coincident with or an accompaniment of relatively high virus concentration. The inclusion bodies, both vacuolate and striated types, are fragile structures disintegrating on contact with the micro-needle or pipette.

FURTHER STUDIES ON THE LONGEVITY AND SWIMMING ABILITY OF SPERMATOZOA

BENJAMIN H. GRAVE

(From DePauw University and the Marine Biological Laboratory, Woods Hole)

A series of experiments designed to measure the total distance which spermatozoa of *Cumingia tellinoides* and *Arbacia punctulata* are capable of swimming, the rate at which they progress, the period of their greatest activity, and their longevity, are described in this paper. They were carried out at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summers of 1931 and 1932.

My indebtedness to Dr. M. H. Jacobs, Director of the Laboratory, for the use of a room and for many conveniences, is gratefully acknowledged.

Apparatus and Methods

Following the publication of a preliminary paper on this subject in 1928 (*Jour. Exper. Zool.*, vol. 51), work was continued with refinements in methods and apparatus used. The construction of the sperm tube as finally perfected is shown in Fig. 1. It may be noted that a slender feed tube (*FT*), inserted at each end, makes it possible to introduce sperm at one end of the tube and eggs at the other and that this method of introducing the sperm reduces to a minimum any disturbance of the water. The elevation (*E*) in the floor of the tube at either end is designed to prevent eggs and sperm from drifting. The two open tubes (*VT*) in the top are intended for ventilation but are probably unnecessary.

In setting up an experiment, the sperm tubes are first filled with sea water which has stood in jars upon the laboratory table over night and which therefore has assumed the temperature of the room and is free from any gases it may have acquired while under pressure in the pipes of the laboratory water system. The sperm tube is then immersed in water of the same temperature with only the feed tubes and ventilating tubes projecting above the surface. For this purpose rectangular aquaria filled with sea water which had also stood on the table over night were used. This undoubtedly insures a uniform environment and avoids convection currents or other disturbing movements of the water as far as possible.

After the sperm tubes, so filled and immersed, had stood undisturbed not less than two hours, one or two drops of eggs were introduced in the feed tube at one end of each sperm tube and two or three drops of a 1 or

2 per cent suspension of sperm at the other.[1] Both eggs and sperm were seen to settle quietly in the feed tubes and to drop vertically from their inner ends, each into the appropriate pocket. It is evident, therefore, that if any of the eggs in the tubes ultimately undergo cleavage they must have been fertilized by spermatozoa that have traversed the full length of the tube. By using tubes of different lengths it was possible to determine not only how far the spermatozoa are able to swim but also the rate of their locomotion.

A possible fault of the former experiments, as reported in 1928, may be found in the fact that the tubes then used were exposed to air and that convection currents might thus be set up within the sperm tubes during the course of the experiment which might therefore have carried the spermatozoa farther than they normally swim of their own effort. However, by carefully shielding the tubes from environmental changes,

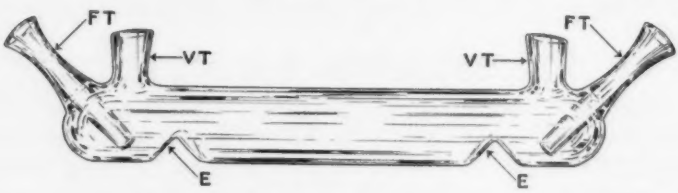


FIG. 1. Type of sperm tube used. *FT*, feed tube; *VT*, ventilating tube; *E*, elevation in the floor of the sperm tube.

including air currents, almost the same results are obtainable by either method. By employing an improved piece of apparatus, and by immersing the tubes in water, this source of error has been eliminated.

METHOD OF CALCULATING THE RATE OF SWIMMING OF SPERMATOZOA

The average normal rate of cleavage of *Arbacia* and *Cumingia* eggs at temperatures of 23 or 24° C. is approximately as follows: first cleavage, 45 to 50 minutes; second cleavage, 70 to 75 minutes; third cleavage, 95 to 100 minutes. By deducting cleavage time from the total elapsed time of the experiment the swimming time of individual spermatozoa for traversing each sperm tube is closely approximated.

[1] Although the initial concentration of the sperm suspensions used in these experiments was relatively high, it became diluted as soon as the spermatozoa began to spread into the water in the body of the sperm tube. The generally recognized influence of the concentration factor on the longevity of sperm may be considered negligible here.

Results

During the summers of 1931 and 1932 numerous experiments of the type just described were made in which approximately constant water temperatures in the sperm tubes were maintained at 22, 23, 24 and 25° C. The time required for spermatozoa to traverse the tubes was repeatedly tested. The results, after the method was perfected, were entirely consistent both in rate of swimming and distance attained. Although spermatozoa were shown to swim for periods of 9 or 10 hours, an unexpected aspect of their activity became evident in that their rate of progress after about 2 or 2½ hours slows down suddenly. A further gradual slowing continues from this point to the end of the life of the sperm. During the latter and greater part of the swimming period the spermatozoa are probably quiescent most of the time, swimming only intermittently. Such intermittent activity, alternating with periods of inactivity, satisfactorily accounts for the observed slowing down in the swimming rate at about the 2-hour interval. These observations on the most active period of spermatozoa are in substantial agreement with other data on the longevity of gametes. Former experiments made to test the longevity of spermatozoa of *Cumingia* and *Hydroides* (*Biol. Bull.*, vols. 54 and 59) have shown that almost 100 per cent fertilization of eggs may be obtained from dilute suspensions of spermatozoa 3 hours old; a small percentage live and are capable of fertilizing eggs 9 to 12 hours after emission, and very few live and function for longer periods.

A comparison of experiments on the gametes of *Cumingia* and *Arbacia* shows that the spermatozoa of the former are somewhat more vigorous than the latter. The spermatozoa of *Arbacia*, with one exception, were observed to swim but 15 to 16 cm. while those of *Cumingia* usually swam 15 to 18 cm.

Results published in a former paper gave the swimming ability of sperm of both species as 28 or 30 cm. It now appears that the extreme limit for even the most vigorous *Cumingia* sperm is 20 cm. Experiments, when performed in tubes exposed to air, frequently give the results originally published. The unexpected difference in the distance covered by spermatozoa in tubes immersed in water and those surrounded by air must be accounted for as the effect of convection currents set up in the water by the more variable temperature environment afforded by air.

Differences in the rate of swimming, as computed from data of recent experiments, show that the spermatozoa of each species are variable in their activity, probably due to differences in vigor. Table I gives the composite results of a series of experiments on the rate of swimming of spermatozoa of *Cumingia* at the temperatures indicated.

The rate at which spermatozoa of *Arbacia* swim these distances are not greatly different from that given in the table. They slow down more quickly than the sperm of *Cumingia* and may require 8 or 9 hours to swim 16 cm. and 4 or 5 hours to swim 15 cm. The rate at which the first 12 cm. is covered is about the same for sperm of the two species. There is a perceptible but not great difference in the rate of swimming of sperm of both species at temperatures of 22 and 25° C. The rate is somewhat slower at the lower temperature and a reduced total swimming distance is also observed. No experiments were made at temperatures lower than 22° C. because of lack of adequate constant temperature apparatus, experience having shown that any considerable change of temperature during the course of an experiment vitiates the results.

Experimental Data

From the twenty-one experiments made during the summer of 1932, one (No. 18) is selected for description as typical. None of these ex-

Table I

Rate of swimming and distance attained by spermatozoa of Cumingia at temperatures of 23 to 25° C.

Length of Sperm Tube	Time Required to Swim the Distance
cm.	
7	40 to 50 minutes
9	50 to 65 minutes
12	1¼ to 1¾ hours
15	1½ to 2½ hours
16	2½ to 5 hours
18	5¼ to 9 hours

periments included less than five sperm tubes and some involved as many as twenty, the tubes varying in length from 7 to 18 cm. Each experiment required from 5 to 9 hours for completion. All of the tubes of an experiment were stocked with sperm from a single individual and with eggs of a single lot. Controls of unfertilized eggs were kept to make sure that they did not cleave because of chance fertilizations. All experiments showed consistent results although the final ones were the most successful because of improvement in technique with experience. Detailed descriptions of additional experiments would show nothing essentially significant or different from those selected.

Experiment 18. July 27, 1932

Sixteen sperm tubes of various lengths were set at 12:00 M. Two drops of sperm and two drops of eggs of *Arbacia* were added to each tube. The records of six of these tubes are given as follows:

Tube No. 1. Length, 7 cm.; temperature, 24.5° C. Eggs examined at 1:40 P.M. Result: Five eggs just cleaving into two cells. Total

time of experiment, 100 minutes; less cleavage time of 45 minutes, swimming time 55 minutes. Sperm swam 7 cm. in 55 minutes.

Tube No. 2. Length, 12 cm.; temperature, 24.5° C. Examined at 3:10 P.M. Result: two eggs in 2-cell stage and one in 8-cell stage. Total time, 190 minutes; cleavage time required to reach 8-cell stage, 94 minutes; swimming time, 95 minutes. (Sperm therefore swam 12 cm. in 1 hour and 35 minutes.)

Tubes No. 3 and 4 were also 12 cm. in length but were kept at a temperature of 22.5° C. (Aquarium placed on a cool water table.) Sperm traversed them in 130 and 135 minutes respectively.

Tube No. 5. Length, 15 cm. Temperature, 24.5° C. Examined at 4:30 P.M. Result: One egg in 4-cell stage, three in 8-cell, and three in 16-cell stage. Total time, 4½ hours. Time required to reach the 16-cell stage, 120 minutes; swimming time, 150 minutes. (Sperm therefore swam 16 cm. in 150 minutes.)

Tube No. 6. Length, 18 cm. Temperature, 24.5° C. Examined at 8:40 P.M. Result: Four eggs in the two-cell stage. Total time 8¾ hours. Swimming time 8 hours.

Comment: This experiment shows that the rate of swimming of the sperm slowed down promptly after 2½ or 3 hours and this was apparent in the whole series of experiments. It also shows that they swam somewhat more rapidly at a temperature of 24.5° C. than at one of 22.5°.

This is the only experiment of the series in which sperm of *Arbacia* swam more than 16 cm. It is probably not exceptional.

Experience has shown that spermatozoa may swim from 2 to 3 cm. after the fifth hour or between the fifth and tenth hours. They make little or no progress after 9 hours.

Discussion

A repetition and reëxamination of the former work extends the data to include the rate of swimming as well as the total distance spermatozoa are capable of swimming and shows that their vigor is rapidly reduced after a relatively brief period of active swimming.

The sharply marked reduction in rate after 2 hours of swimming, as shown by Table I, is significant. It probably means that although spermatozoa live and function for much longer periods, their vitality and energy are practically spent within 2½ hours. There seems also to be a great difference in the vitality of the sperm of different individuals of the same species.

The first spermatozoa to arrive at the opposite end of the sperm tubes and therefore the first to fertilize eggs have been considered as

giving the rate of swimming. As a matter of fact, larger numbers of spermatozoa arrive later, and others continue to arrive for several hours, as shown by the fact that certain 12 cm. tubes which were allowed to remain undisturbed for 10 hours contained every stage of cleaving eggs from two cells to the blastula. This also shows that spermatozoa continue to swim more or less for a period of 9 or 10 hours.

Spermatozoa which have been stimulated to great activity and weakened by hours of swimming may become quiescent and lie almost motionless. If these spent spermatozoa are now brought into the presence of eggs, or of sea water which has contained eggs for some time, they often revive and again swim actively. It is possible that spermatozoa are attracted by emanations from the eggs and that they are directed in their swimming by chemotaxis. The conditions of our experiments make it unlikely that stimuli act through any considerable distance, and it may be assumed that we are measuring only the activity of spermatozoa as stimulated by normal sea water. If, therefore, the direction of swimming of the spermatozoa is at random and without orientation it is probable that a spermatozoön which crosses from one end of a sperm tube to the other in the course of several hours may actually have travelled a greater distance than the length of the tube, especially if it reversed the direction of its locomotion several times in the process. There is no way to ascertain or measure this sinuous path. Hence the length of the tube is taken as the distance attained. Ignoring these complications, it is concluded that vigorous spermatozoa of certain marine invertebrates may swim 20 cm. in sea water and that they attain three-fourths or four-fifths of this distance during the first 3 hours after extrusion into sea water.

BIBLIOGRAPHY

GEMMILL, J. F., 1900. On the Vitality of the Ova and Spermatozoa of Certain Animals. *Jour. Anat. and Physiol.*, **34** (N. S., **14**): 163.

GRAVE, B. H., AND R. C. DOWNING, 1928. The Longevity and Swimming Ability of Spermatozoa. *Jour. Exper. Zool.*, **51**: 383.

GRAVE, B. H., 1928. Vitality of the Gametes of Cumingia tellinoides. *Biol. Bull.*, **54**: 351.

GRAVE, B. H., AND J. F. OLIPHANT, 1930. The Longevity of Unfertilized Gametes. *Biol. Bull.*, **59**: 233.

HOYT, W. D., 1910. Physiological Aspects of Fertilization and Hybridization in Ferns. *Bot. Gaz.*, **49**: 340.

LILLIE, F. R., 1914. The Mechanism of Fertilization in Arbacia. *Jour. Exper. Zool.*, **16**: 523.

LILLIE, E. R., 1923. The Problems of Fertilization. Chicago University Press.

ON THE REALITY OF SPINDLE FIBERS

FRANZ SCHRADER

DEPARTMENT OF ZOÖLOGY, COLUMBIA UNIVERSITY, NEW YORK CITY

It is a striking fact that our knowledge of chromosome structure and behavior is far in advance of what we know about the rest of the mitotic figure. In spite of the interest of earlier workers in the mitotic spindle, we have very little definite information about it and as proof of this may be mentioned the fact that there is still disagreement on such a basic point as to whether the so-called spindle fibers have a morphological reality in the living spindle. It is this latter question with which I am here concerned.

For the sake of clarity it may be well to indicate the structures that are involved. They were recognized by some of the earliest cytologists and though their exact nature may not be clear, it is nevertheless patent that on a topographical basis alone one may distinguish three types of fibers in the fixed anaphase spindle: (1) The primary or continuous fibers that usually extend from pole to pole and do not establish any contact with the chromosomes; (2) the half-spindle components each of which is connected with a chromosome on the one hand and one of the poles on the other; (3) the interzonal connections, which stretch between the separating daughter chromosomes (text fig. 1).

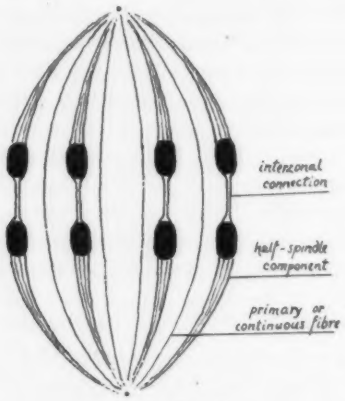
THE PRESENT STATUS OF THE QUESTION

That the spindle as a whole is morphologically differentiated from the general cytoplasm has been amply demonstrated by such work as that of Chambers and Sands (1923) and is indeed quite evident from an examination of the living cell. The question at issue is whether the fibrous structures seen in fixed preparations have some morphological representation in the living spindle, or whether the latter is quite homogeneous.

Naïvely expressed, it is evident that something must be responsible for the structures seen in fixed spindles. So far as I can see, only two alternative explanations are possible. One is that spindle fibers represent lines of force which, although invisible in the living spindle, bring about a differential coagulation when the latter is fixed. Such a coagulation results in the longitudinal streaks or lines that we call fibers. In other words, the proponents of this view maintain that in the unfixed spindle there is no morphological basis for such fibers. In contradistinction, the alternative view is that the living spindle is not

homogeneous and that the fibers seen in fixed cells are present in some morphological form also when the cell is alive.

However, the issue is complicated by the fact that some early and much recent evidence indicates that the spindle is not to be regarded as a unit and that, as I have argued in an earlier publication (1932), the three spindle elements are not necessarily related to each other in any basic way. Since the material used in the present investigation is not favorable for work on the primary or continuous fibers, I will venture no conclusion concerning them. On the other hand, the interzonal connections can hardly be regarded any longer as a subject of the question here involved, since their actuality now seems sufficiently well established. In substantiation I need mention only the work of Němec (1929 and earlier), who was able to separate the phragmoplast from



TEXT FIG. 1

the nuclei by centrifuging and other means and still found the orthodox fibers appearing in it on subsequent fixation; the report of Ellenhorn (1933) that interzonal connections (which, like me, he interprets as tubes) are visible in certain living anaphases; and finally, the persistence of interzonal fibers to the metaphase and anaphase of the succeeding division (Schrader, 1932).

But no such definite evidence is available for the half-spindles and it is to them that my main attention is directed. Speaking generally, the case for a homogeneous structure of the half-spindle is based on the three following arguments:

1. In the normal, living cell the half-spindle shows no fibrous structure and appears perfectly homogeneous.

2. Chambers (1924) reports that when a microdissection needle is inserted into the half-spindle region of a living cell and moved to and fro, no corresponding movements occur among the chromosomes. Again, if a chromosome is pulled out of the metaphase plate it shows no fiber adhering to it. He argues that this must indicate the absence of any morphological connection between chromosome and pole.

3. Some cytologists, especially certain members of the school of Gregoire, believe that after good fixation spindle fibers appear in the stained preparation no more than they do in the living spindle.

The first of these arguments may well be admitted as a weighty one. So far as I know no one has ever demonstrated conclusively that fibers may be seen in the living spindle. I can only suggest, as many have done before me, that the indices of refraction of fibers and ground substance may be so close to each other that an optical distinction becomes impossible under ordinary conditions.

The evidence brought forward by Chambers and so frequently referred to, can hardly be considered as decisive. As Bělař (1929*a*) has already pointed out, we know almost nothing about the resistance that might be offered to the needle by the fibers. Nor do we have any grounds to believe that a fiber pulled out into the cytoplasm is a conspicuous object. At best, Chambers neglected to take the obvious step of fixing and staining the cells thus operated upon. Their appearance then would, of course, be of great importance.

The claim that in perfectly fixed cells, and especially those at the periphery of the tissue, no spindle fibers appear, may well be taken seriously when it is advanced by such careful workers as Robyns (1929 and earlier) and Bleier (1930 and 1931). But the claim is not easily put to a test. It must be remembered, however, that good fixation does not necessarily imply favorable staining properties. That peripherally located cells are fixed more perfectly than more deeply lying ones is almost universally accepted, but I would like to point out that such cells frequently stain imperfectly. Thus the cells at the periphery of the heteropteran testis are frequently more rapidly destained than those nearer the center; and in the case of such Amphibia as *Amphiuma, Batrachoseps,* and *Plethodon,* these peripheral cells very often never accept such stains as saffranin and hæmatoxylin at all. That applies especially to chromosomes in the prophase stages as well as the spindle elements, and of the morphological reality of the former there can, of course, be no doubt. In short, a good fixation does not in itself guarantee the appearance of all cell organs after staining, the latter depending on additional factors, chief of which appears to be the hydrogen ion concentration (Seki, 1933).

To sum up, the evidence against a structural reality of half-spindle fibers is not complete. But a similar criticism has been made of the case for the other side of the question. The evidence there, usually indirect, is exemplified by such investigations as those of Bělař (1929*a* and *b*) and Schaede (1930). These workers produced, the one by immersion in hypertonic media, the other by centrifuging, splits in the spindle. These splits always occur in or parallel to the long axis and both investigators concluded that, conservatively speaking, there must be a structural differentiation, longitudinally oriented, in the living spindle. To this Bleier (1931) has objected that if through such external agencies splits arise in a homogeneous body under bipolar tension, they must of necessity take the general direction of such tension and do not indicate a preëxisting longitudinal structure per se. He makes similar criticisms of some others of Bělař's ingenious arguments and though I do not concede that he completely disposes of the latter, it is evident that the case is still open.

In view of this rather unsatisfactory status of the question, it is strange that so little attention has been paid to a type of evidence that has been in the literature for some time. This evidence pivots on the simple argument that if spindle fibers can be bent in the living cell, they can not be interpreted simply as lines of force. Such bending has been described as resulting from natural or at least unknown causes by Bonnevie (1906) and others; it has been observed as resulting from the cell distortions involved in the effects of hypertonic media (Bělař); and most frequently of all it has been reported as consequent on the centrifuging of dividing cells (among others, Andrews (1915), Lillie (1909), Morgan (1910), Spooner (1911), and Schaede (1930)).

It will be recognized at once that the bending of the half-spindle fibers becomes significant only if it can be established that the experimental treatment had not already induced coagulation when the bending occurred. This implies that controls similarly treated must show that such cells continue to live and divide. Such controls are manifestly impossible in naturally occurring distortions. The effects of hypertonicity are not conclusive because Bělař states that, whereas anaphases may recover, metaphases so treated almost never do. Unfortunately, it is the latter in which we are certainly concerned with half-spindle fibers, and therewith the objection becomes possible that the fibrous structure reported by Bělař was first produced by the hypertonic medium and then bent and distorted by continued action of the latter.

The evidence from centrifuging suffers because none of the workers distinguish sharply between half-spindle fibers and interzonal connections and, indeed, most of their figures deal with the latter. However,

Lillie speaks of serious disarrangement of fibers and shows in Fig. 7 of his paper a metaphase which is not very striking, and Morgan remarks on and shows in Fig. 32 of his paper a slight bending of the half-spindles in a metaphase. The best evidence is contained in the paper by Spooner, who reports that spindle fibers may be bent without permanent injury to the cell and illustrates this effect especially in Fig. 3 of her paper, where two metaphases show bending of the half-spindles.

I can conceive of only one loophole in the argument. If the surface of the spindle body is, let us say, negatively charged, and the hypothetical lines of force are under its influence, then a bending of the spindle surface might possibly result in a corresponding deflection of the interior lines of force. The consequent fixation would then form fibrous coagulations which, in conformation to the lines of force, would be bent. If, however, the centrifuged spindle shows fibers that are not only curved but in addition show some displacement with respect to each other, this last objection would fall to the ground. Miss Spooner's figures are too sketchy to allow of a judgment on the point and I therefore undertook the rather thankless task of once more covering the ground.

Material and Methods

An Emerson centrifuge with 10,000 revolutions per minute and an 8 cm. radius was used throughout. The centrifuge has the advantage of reaching full velocity within 3 or 4 seconds and coming to a full stop within the same length of time.

The following material was used:

Heteroptera: testes of *Alydus eurinus, Brochymena arborea,* and *Euschistus fissilis.* Fixation: strong Flemming.

Mollusca: eggs of *Planorbis glabratus* in first and second cleavage. Fixation: Bouin.

Crustacea: testes of *Homarus americanus.* Fixation: strong Flemming. Eggs of several species of *Cyclops* in early cleavage stages. Fixation: Carnoy or Carothers.

Heidenhain's hæmatoxylin method was used in staining and all preparations were mounted in Euparol.

Heteroptera

Testes of *Alydus* were centrifuged ½, ¾, 1, 2, and 3 minutes; testes of *Brochymena* and *Euschistus* for 1, 2, 3, 5, 7, and 9 minutes. One testis of each specimen was fixed immediately after centrifuging while the other was retained as a control in modified Ringer's solution. The remarkable result was that none of the metaphases even when

centrifuged for 9 minutes showed a significant distortion. The interzonal fibers of the anaphases, on the other hand, already showed bending and twisting effects at 3 minutes. This fact, of course, can only emphasize that a distinct difference must exist between the two spindle regions. It was found, however, that whereas uncentrifuged testes remain normal in the Ringer's fluid for 7 or 8 hours, testes centrifuged for 5 minutes or longer already show evidence of degeneration after about four hours. Such cells show a characteristic granular deposit in the cytoplasm and frequently small vacuoles make their appearance. Deleterious effects are thus produced in material centrifuged for such periods, even though in material fixed immediately no detrimental results are to be observed.

Mollusca

After centrifuging (from 1 to 3 minutes), the egg masses of *Planorbis* were divided into two portions, one to be fixed at once and the other to be kept as control in ordinary pond water. In spite of the stratification of the egg materials, development occurred normally in all the centrifuged controls, as Morgan and Conklin have already reported in other mollusks. However, metaphase spindles were only sporadically affected by centrifuging, the cases of bent half-spindles occurring with about equal frequency for all the periods used. Apparently such bending results only when a mass of yolk globules becomes accidentally lodged on one side of the spindle and it is therefore not the direct result of centrifuging. In no such instance was the effect very striking (Fig. 4).

Crustacea

Homarus

From testes with spermatocyte divisions portions approximately half a centimeter long were cut out, the controls being derived from the topographically corresponding region of the other branch of the testis. The material was centrifuged for ½, 1, 1½, 2, and 2½ minutes. Controls

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Plate I

1. *Homarus.* Normal metaphase of spermatocyte division.
2. *Homarus.* Spermatocyte metaphase centrifuged for 1½ minutes, showing two broken components in the lower half-spindle and a chromosome with its components dislodged on the right side.
3. *Homarus.* Spermatocyte metaphase or early anaphase showing extreme distortion after being centrifuged for 1½ minutes.
4. *Planorbis.* Metaphase of first cleavage in the egg, centrifuged for 2¼ minutes. Note yolk globules on left side of spindle.
5. *Cyclops americanus.* Telophase of third cleavage in egg centrifuged for one minute, showing distorted interzonal connections.

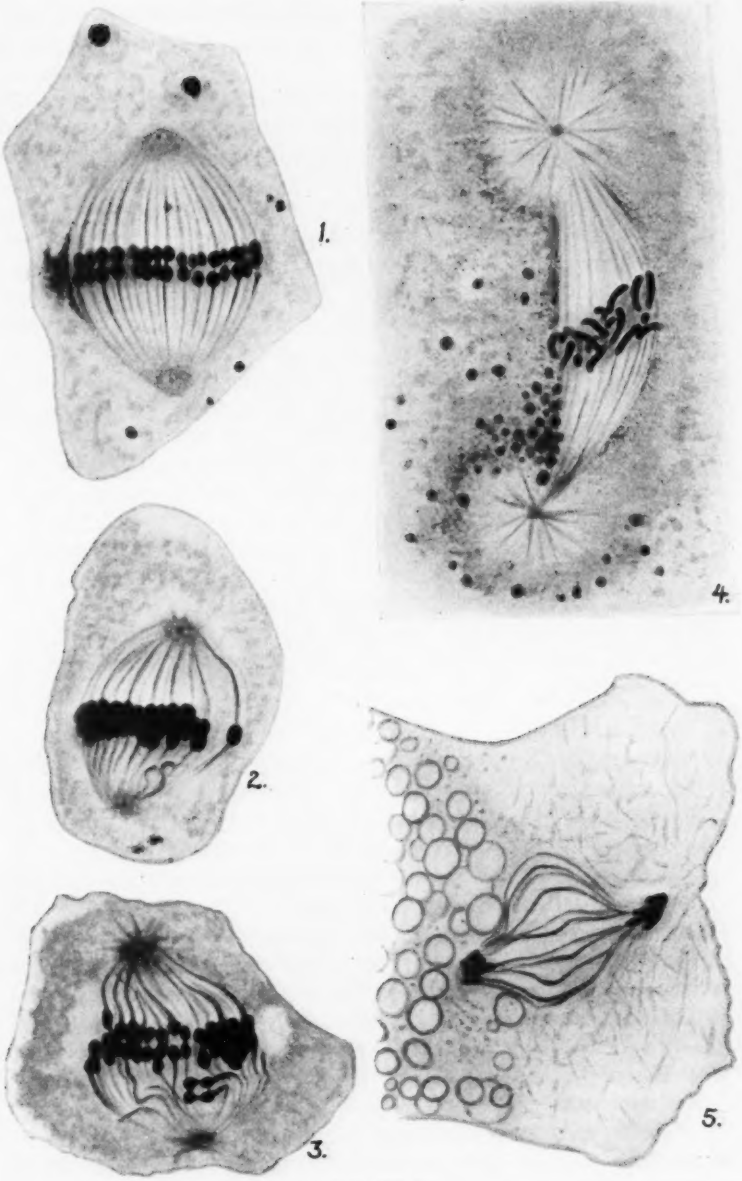

PLATE I

were kept in Ringer's fluid or in sea water sterilized with heat. I found it impossible, however, to avoid bacterial action which generally set in after 5 or 6 hours.

Mention must be made of the fact that spindle fibers of uncentrifuged spermatocyte cells show a certain amount of crinkling, the direct effect of fixation shrinkage. But this effect is readily distinguished from the more extreme distortion resulting from centrifuging.

Centrifuging for 1½ minutes produces effects such as are shown in Figs. 2 and 3. It will be seen that not only are the fibers distorted, but they are shifted with respect to each other and not infrequently torn out of the main body of the spindle. The case of *Homarus* would thus be decisive in establishing an actuality of half-spindle fibers if the cells involved would through recovery attest that coagulation had not occurred. Unfortunately this can not be definitely established. Controls examined three hours after centrifuging, indeed, show that in many regions the cells appear normal, but in other, rather sharply restricted regions of the same tissues a definite degeneration is evident. The distortion effects already described are found in similarly restricted regions and I can only suggest that we are dealing here with the results of a crumpling and folding within the narrow confines of the centrifuge tube. It is very likely the pressure incident to such folding that is to be held responsible for such effects and of course also for the later degeneration. But in view of the likelihood that degeneration occurs in cells with bent spindles, the material becomes useless for our purpose and was reluctantly abandoned.

Copepoda

The two egg masses carried by females of various species of *Cyclops* are almost ideal for the purposes of the experiment. In the two species used, each mass contains about a dozen eggs and these, at least during early cleavage, are all in practically the same phase of mitosis. The egg masses easily can be detached from the mother's body and continue to develop normally in ordinary pond water.

In centrifuging, both egg masses of a female were used, one being fixed immediately afterward while the other was kept in pond water for various lengths of time before fixation. All the drawings were made from *Cyclops americanus,* which was readily obtained in the spring. But with the beginning of June this species became scarce and for purposes of studying the effect of centrifuging on the time of mitosis, *Cyclops signatus* was used.

It should be remarked that here, as in *Homarus* and *Planorbis,* the effect of centrifuging seems to be an indirect one. If the spindle, as sometimes happens, lies parallel to the stratification of yolk and other

materials, it appears to be affected very little. But usually it has a diagonal position so that one pole becomes pushed into or against the stratified yolk while the other is under pressure from the peripheral portion of the egg. It is chiefly in such cases, which comprise the great majority, that the effects to be described are encountered.

The normal, uncentrifuged metaphase of *C. americanus* is shown in Fig. 6. The distance between the poles of the early cleavage figures is unusually great. The half-spindle fibers are sharply marked and stain rather heavily for a part of the distance between the chromosomes and the pole, but then they suddenly become fainter and can hardly be traced near the tip of the spindle. The astral center appears to be at some distance from this tip. The fully formed half-spindle as a whole is plainly demarked from the cytoplasm and though it is difficult to show in drawing, there appears to be a matrix or ground substance which occupies the spaces between the fibers or components.

Centrifuging for one minute frequently produces distortion of the metaphase spindle, but the effect is more or less sporadic. The interzonal connections of the anaphase are, however, nearly always distorted in such eggs, the result depending on the position of the mitotic figure at the time of centrifuging. Thus the group of interzonal fibers may be bent as a whole if the two groups of chromosomes are shifted with respect to each other, or else they may be kinked and separated if the centrifugal force coincides with the long axis of the spindle in its direction (Fig. 5). Centrifuging for 1¼ and 1½ minutes causes similar if not greater effects on interzonal fibers, but it also is more constant in its effects on the half-spindles. Since centrifuging for 1¾ minutes did not markedly increase such changes, nearly all the observations were made on eggs centrifuged for 1¼ or 1½ minutes.

It will be remembered that in the early divisions of the copepod egg there is a striking gonomery. This side by side association of maternal and paternal nuclei in a double spindle is easily disturbed by centrifuging,—one spindle often being shifted on the other. But generally this is done through bending of the poleward or distal region of the half-spindle without severing connection with the asters. This region seems to offer little resistance, whereas the more sharply outlined parts of the half-spindle components proximal to the chromosomes are evidently stiffer (Figs. 7 and 10). Occasionally, however, the two spindles become completely separated at one pole, although in my experience in every such case the connections with the opposite pole were maintained.

In two or three instances the spindles show a splitting or separation of the components without any marked bending. Such a phenomenon has already been described by Bělař (1929) and more particularly

Schaede (1930), and will not be especially mentioned in the present work (Fig. 10).

It is with the more common cases of distortion that I am particularly concerned. That the half-spindle fibers are relatively stiff has already been remarked, but bending nevertheless occurs even in the proximal region. It is to be emphasized that the components of a half-spindle are not only bent as a group, but that bending occurs unequally so that even adjacent ones may show varying degrees of distortion and displacement (Figs. 7, 8, and 9). However, it is rarely that a fiber or component is so dislodged as actually to protrude from the general spindle body, a result which is so common in *Homarus*. It must be obvious that no system of lines of force would show reactions of the type just described.

The possible criticisms of such a demonstration of the actuality of spindle fibers have already been referred to. To check the possibility that centrifuging first coagulates a homogeneous spindle substance in fiber-like streaks and then, being continued, produces the bending here described,—centrifuged metaphases were studied in vivo. If the visibility of fibers depends on coagulation in a homogeneous substance, and if such coagulation can be induced by centrifuging, then fibers should be seen in freshly centrifuged, unfixed metaphases. The spindles are found without much difficulty, but though they are studied more readily than uncentrifuged figures (because centrifuging removes the obscuring yolk spheres), it is not often that an exact side view is encountered. In none of the four instances where this was done could any definite fibrous structure be identified and except for the absence of yolk the appearance did not seem to differ from that of uncentrifuged spindles. Evidently, a coagulation of the ordinary sort is not involved.

A more extensive study was made of the effects of centrifuging on subsequent activity of the egg. In normal and uncentrifuged eggs of *Cyclops signatus* it takes very close to three-quarters of an hour to com-

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PLATE II

(All figures of *Cyclops americanus*. In Figs. 6, 8, and 9 only one member of the double spindle appears)

6. First cleavage metaphase in normal, uncentrifuged egg.

7. Second cleavage metaphase, centrifuged for 1¼ minutes. Showing a shifting of the two gonomeric spindles on each other, with compensating bends in the half-spindles near the poles.

8. First cleavage metaphase, centrifuged for 1¼ minutes. Showing that half-spindle components can be bent independently of each other.

9. First cleavage metaphase, centrifuged for 1¼ minutes. Showing that half-spindle fibers can be bent independently of each other.

10. Second cleavage metaphase centrifuged for 1¼ minutes. Showing splitting in one of the gonomeric spindles and bending of the polar end of the half-spindle.

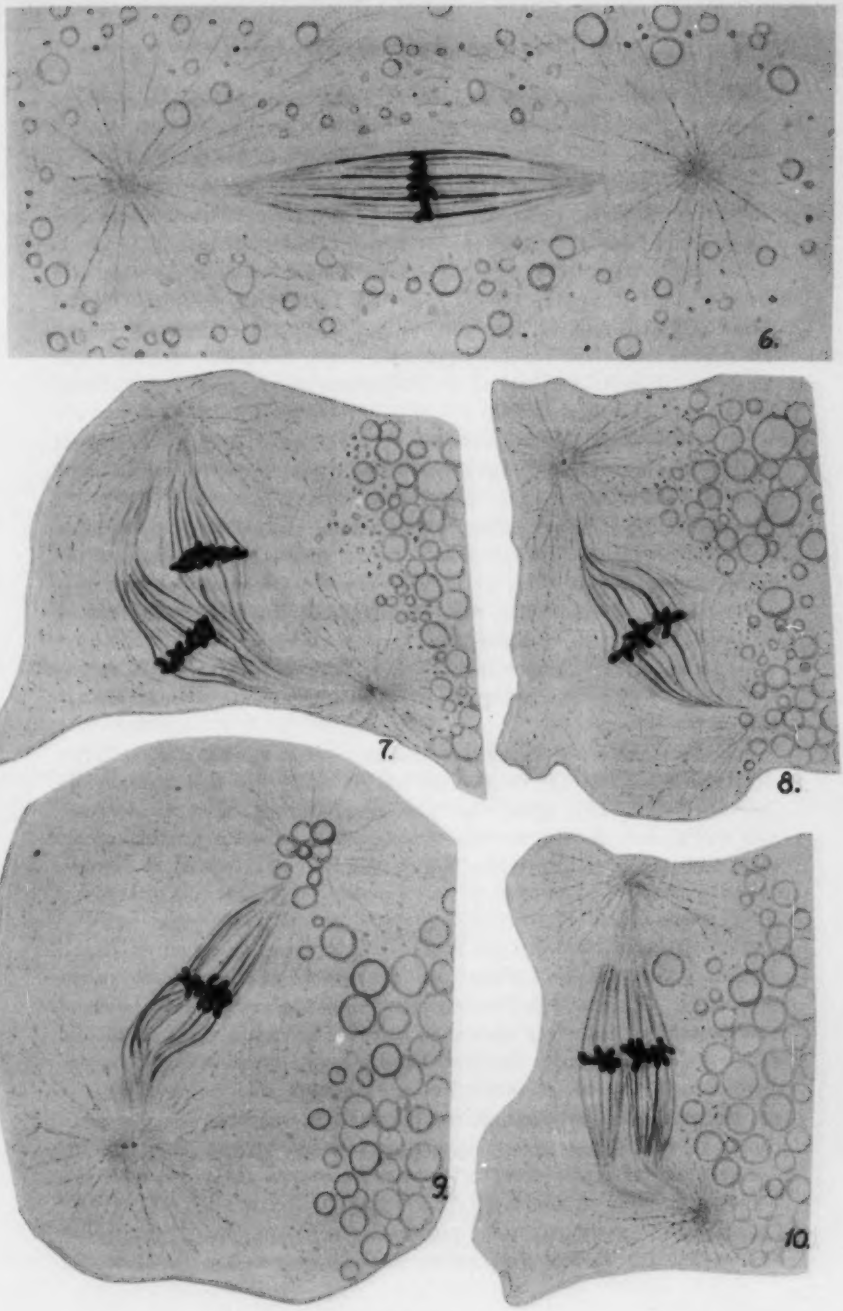

PLATE II

plete an early cell cycle, i.e., from metaphase to metaphase. Eggs centrifuged when in the first or second metaphase and fixed one-half hour afterward had reached the middle prophase of the succeeding division (12 eggs); after three-quarters of an hour the eggs had reached an early metaphase (with a formed spindle but some trace of the disintegrating nuclear membrane) of the division following (11 eggs); after one hour an early prophase of the second following division had been reached (6 eggs); after 2 hours the succeeding two divisions had been completed and the prophase of the third had been initiated (about 20 eggs); and after 5 hours eggs centrifuged when in the first metaphase had attained the stage in which blastula formation is begun (about 30 eggs).

It will be seen that the duration of a cell cycle in eggs centrifuged during first or second metaphases is thus not markedly different from that in uncentrifuged eggs, that is, about three-quarters of an hour. The possibility of some slowing nevertheless remains, not only because it is difficult definitely to limit the various phases but also because the metaphase appears to be of some duration and it is not possible to recognize whether one is dealing with one that has just been established or one that is about to be terminated for the anaphase. But this period does not exceed 10 minutes at best and is more likely to be in the neighborhood of 5 minutes. A lag due to centrifuging thus cannot be greater than a few minutes.

In short, the centrifuging either brought about no delay in the mitotic activities or else the latter were caused to lag for only a negligible space of time. This again must mean that no coagulation is involved, and this in turn implies that the fibrous or at least longitudinal structures were present in the living half-spindle.

Conclusion

Throughout the preceding pages the word "fiber" has frequently been used. It must be understood that it is employed for convenience only since there is every indication that so far as the half-spindle is concerned the structures involved have a greater complexity than the term "fiber" would indicate. Indeed, the same may be said for the interzonal connection although, as I have tried to show in a previous paper, the half-spindle and interzonal elements show some very definite differences from each other. The case for such a difference certainly becomes stronger in the light of Ellenhorn's recent observations and finds still further support in the present investigation, where the relative ease with which the interzonal connections are distorted in the Heterop-

tera and Copepoda stands in distinct contrast to the behavior of the half-spindle components under identical experimental conditions.

However, so far as the present work is concerned, the detailed structure of these elements is not involved. To repeat, the question here is simply whether the living spindle is homogeneous or whether there is a morphological basis for the half-spindle components, as they appear in fixed preparations. I consider that the evidence can point only to the last-named of these alternatives.[1]

I must concede, however, that I have not been able to overcome an objection that has been expressed in connection with the argument of Bělař and Schaede, that longitudinal splits in the treated spindle presuppose a corresponding structure in the normal spindle. This objection is voiced by Bleier (1931) as follows (freely translated): "From such splits one could more logically deduce that the main spindle is composed of smaller spindles (Teilspindeln)." It so happens that the spindles of copepod eggs are particularly open to this objection, if such it be. I adduce this not only from direct observation (especially of the maturation figures) but also from the readiness with which the chromosomes are separated from each other experimentally, each accompanied by a tiny spindle (Schiller, 1909). The close relationship of such spindle structure to that seen in *Acroschismus* (Hughes-Schrader, 1924), where the compound nature is evident in normal, untreated spindles, is too obvious to merit further comment.

But whether these small, component spindles are called Teilspindeln or whether they are called half-spindle components as I have done, seems to me of little import. That they are more firmly linked together in some forms than in others, is clear, but even in such a closely knit spindle as that of the Heteroptera the similarity of half-spindle components to tiny individual spindles is so striking as to raise the question whether all spindles are not basically composed of Teilspindeln. It is true that in many, perhaps most, cases the finished metaphase spindle seems to present still another and homogeneous substance which occupies the spaces between the half-spindle components, but if this is present in such cases as *Acroschismus* and *Llaveia,* it must arise quite secondarily.

Indeed, if Bleier could commit himself more definitely to the implication in the statement quoted, he and I might well find ourselves on

[1] It is of interest to note that Belling apparently was also convinced of the reality of the half-spindle components, for referring to some unpublished work he wrote (1933, p. 80): ". . . 'Traction' fibers of the spindle are real, I consider, in *Lilium.* I have, by starving, got them to show in a vacuole."

Unfortunately nothing beyond this short statement is given. It is hoped that the manuscript pertaining to the work in question is in condition to be published.

common ground—to wit, that the half-spindle is primarily composed of smaller elements which have hitherto been called fibers but which I have termed more noncommittally " components."

Summary

1. By centrifuging, the fibers or components of the half-spindle are bent and distorted, not only as a group but also independently of each other.

2. Metaphases so treated continue their mitotic activities and it is concluded that the half-spindle components as seen in fixed preparations are not coagulation artefacts but have a morphological basis in the living spindle.

3. The experiments lend further support to the claim that half-spindle components and interzonal connections differ from each other in structure and certain other physical properties.

REFERENCES CITED

Andrews, F. M., 1915. Die Wirkung der Zentrifugalkraft auf Pflanzen. *Jahrb. wiss. Bot.*, **56**: 221.

Bělař, K., 1929*a*. Beiträge zur Kausalanalyse der Mitose. II. Untersuchungen an den Spermatocyten von Chorthippus (Stenobothrus) lineatus Panz. *Arch. f. Entw.-Mech.*, **118**: 359.

Bělař, K., 1929*b*. Beiträge zur Kausalanalyse der Mitose. III. Untersuchungen an den Staubfadenhaarzellen und Blattmeristemzellen von Tradescantia virginica. *Zeitschr. f. Zellf.*, **10**: 73.

Belling, J., 1933. Critical Notes on C. D. Darlington's " Recent Advances in Cytology." *Univ. Calif. Publ. Bot.*, **17**: 75.

Bleier, H., 1930. Untersuchungen über das Verhalten der verschiedenen Kernkomponenten bei der Reduktionsteilung von Bastarden. *La Cellule*, **40**: 83.

Bleier, H., 1931. Zur Kausalanalyse der Kernteilung. *Genetica*, **13**: 27.

Bonnevie, K., 1906. Untersuchungen über Keimzellen. I. Beobachtungen an den Keimzellen von Enteroxenos östergreni. *Jena. Zeitschr. Naturw.*, **34**: 229.

Chambers, R., 1924. General Cytology. (Edited by Cowdry.) Page 272.

Chambers, R., and H. C. Sands, 1923. A Dissection of the Chromosomes in the Pollen Mother Cells of Tradescantia virginica L. *Jour. Gen. Physiol.*, **5**: 815.

Conklin, E. G., 1917. Effects of Centrifugal Force on the Structure and Development of the Eggs of Crepidula. *Jour. Exper. Zool.*, **22**: 311.

Ellenhorn, Jakob, 1933. Experimental-photographische Studien der lebenden Zellen. *Zeitschr. f. Zellf.*, **20**: 288.

Hughes-Schrader, Sally, 1924. Reproduction in Acroschismus wheeleri Pierce. *Jour. Morph.*, **39**: 157.

Lillie, F. R., 1909. Karyokinetic Figures of Centrifuged Eggs; an Experimental Test of the Center of Force Hypothesis. *Biol. Bull.*, **17**: 101.

Morgan, T. H., 1910. Cytological Studies of Centrifuged Eggs. *Jour. Exper. Zool.*, **9**: 593.

Němec, B., 1915. Einiges über zentrifugierte Pflanzenzellen. *Bull. internat. Acad. Tchéque Sci. Boheme*, vol. 20.

NĚMEC, B., 1927. Über die Beschaffenheit der achromatischen Teilungsfigur. *Arch. exp. Zellf.*, **5**: 77.

NĚMEC, B., 1929. Ueber den Einfluss des Nikotins auf sich teilende Zellen. *Protoplasma*, **7**: 99.

ROBYNS, W., 1924. Le fuseau de caryocinèse et le fuseau de cytocinèse dans les divisions somatiques des Phanérogames. *La Cellule*, **34**: 365.

ROBYNS, W., 1929. La figure achromatique sur matérial frais, dans les divisions somatiques des Phanérogames. *La Cellule*, **39**: 83.

SCHAEDE, R., 1930. Zentrifugalversuche mit Kernteilungen. *Planta*, **11**: 243.

SCHILLER, I., 1909. Über künstliche Erzeugung "primitiver" Kernteilungsformen bei Cyclops. *Arch. f. Entw.-Mech.*, **27**: 560.

SCHRADER, FRANZ, 1932. Recent Hypotheses on the Structure of Spindles in the Light of Certain Observations in Hemiptera. *Zeitschr. wiss. Zool.*, **142**: 520.

SEKI, MASAJ, 1933. Zur physikalischen Chemie der histologischen Färbung. VIII and IX. *Zeitschr. f. Zellf.*, **18**: 1.

SPOONER, GEORGINA B., 1911. Embryological Studies with the Centrifuge. *Jour. Exper. Zool.*, **10**: 23.

OBSERVATIONS ON THE GENUS STERRHURUS LOOSS, WITH A DESCRIPTION OF STERRHURUS BRANCHIALIS SP. NOV. (TREMATODA, HEMIURIDÆ)

H. W. STUNKARD AND R. F. NIGRELLI

(From the Department of Biology, New York University, and the New York Aquarium)

The genus *Sterrhurus* was erected by Looss (1907*a*) with *S. musculus* as the type species. In the genus he included also *S. imocavus* Looss, *S. grandiporus* (Rudolphi, 1819) [= *Dist. grandiporum* Rud., = *Lecithochirium grandiporum* (Rud.) Lühe, 1901], and *S. fusiformis* (Lühe, 1901) [= *Lecithochirium fusiforme* Lühe]. Looss included the genera *Sterrhurus, Lecithochirium* Lühe, 1901, *Synaptobothrium* von Linstow, 1904, *Plerurus* Looss, 1907, and *Brachyphallus* Odhner, 1905 in the subfamily Sterrhurinae. Fuhrmann (1928) listed *Synaptobothrium* as a synonym of *Lecithochirium* and transferred *Brachyphallus* to the subfamily Hemiurinae. Recently, Manter (1934) added the new genera *Dinosoma* and *Parasterrhurus* to the subfamily Sterrhurinae.

In a more extended paper, Looss (1907*b*) discussed the morphology of the hemiurid trematodes and their taxonomic relations. He gave a detailed account of the characteristic features of the genus *Sterrhurus,* and brief specific diagnoses of the four species listed above. Subsequently seven other species have been added to the genus. Two of them, *S. monticellii* and *S. lævis,* had been described by Linton (1898) and assigned to the genus *Distomum. D. monticellii* was described from the stomach and gills of *Remora remora* and later Linton (1901) also reported the parasite from the intestine of *Pomatomus saltatrix* and *Paralichthys dentatus.* Linton (1905) reported both immature and gravid worms, all of which were listed tentatively as *D. monticellii,* from nineteen different species of fish at Beaufort, North Carolina. Often only a single specimen, and in other cases only a few of them were found in each host. The species was recorded in his (1907) studies on the parasites of Bermuda fishes, and, in his account of the trematodes of the Dry Tortugas, Linton (1910) placed it in the genus *Sterrhurus.* Linton (1898) also described *Distomum læve* from *Macrourus bairdi.* Looss (1899) classified this worm as *Hemiurus lævis* (Linton) and Manter (1931) transferred it to the genus *Sterrhurus.* Another species, *Sterrhurus brevicirrus,* was described by Nicoll (1915) from the intestine of *Pomadasis hasta.* Manter (1934) redescribed *S. lævis* (Linton) and added the new species, *S. floridensis, S. præclarus, S. ro-*

bustus and *S. profundus*. Nigrelli and Stunkard (1933) reported a species of *Sterrhurus* from the gills of the cutlass fish, *Trichiurus lepturus* Linn. Further study of this material shows the specimens to be specifically distinct from all previously described forms and for them the name *Sterrhurus branchialis* is proposed.

Sterrhurus branchialis sp. nov.

More than three hundred sexually mature worms were taken from the gills and seven others were found in the intestine of a single individual of *Trichiurus lepturus*. The fish was taken near Long Island, New York, although the natural habitat of the species is the deep water of the warm Atlantic, extending north to Virginia and occasionally as far as Massachusetts.

Fixed and stained specimens are 0.76–2.5 mm. long and 0.34–0.57 mm. wide. The body is only slightly flattened, wall strongly developed, smooth when the specimen is fully extended but thrown into numerous fine annulations when the worm is contracted. The cuticula in the anterior part of body is 0.003–0.009 mm. in thickness. The "tail" is much narrower than the body proper, when fully extended about one-third the body length. It is filled with homogeneous, chromophile globules (presumably excretory products) which in living worms were observed to pass to the exterior through the pore at the tip of the "tail." The acetabulum is 0.2–0.4 mm. in diameter, situated 0.26 to 0.72 mm. from the anterior end, depending on the degree of contraction in this portion of the body. The dorsal lip is present and may show two or three weakly-muscular lobes or papillæ.

Digestive System. The oral sucker is 0.11–0.16 mm. in diameter. The pharynx is immediately behind and about three-fifths as large as the oral sucker. The esophagus is short; intestinal rami extend to the posterior third of the body and enter the "tail" only when the animal is strongly contracted. The distal ends of the ceca are slightly dilated.

Male Reproductive System. The testes are somewhat lobed, spherical to oval, the long axis lateral in contracted specimens, 0.11–0.235 mm. in diameter. They are postacetabular, usually oblique, sometimes symmetrical (especially in immature, small, and contracted individuals). Ducts from the testes unite dorsal to the acetabulum to form the S-shaped seminal vesicle. The initial portion of the vesicle is large, saccate, followed by the anterior curved portions. The anterior part is dorsal to the sinus sac and a short recurved duct, the pars prostatica, connects the seminal and prostatic vesicles. The terminal half of this duct is widened and opens at an angle into the prostate vesicle.

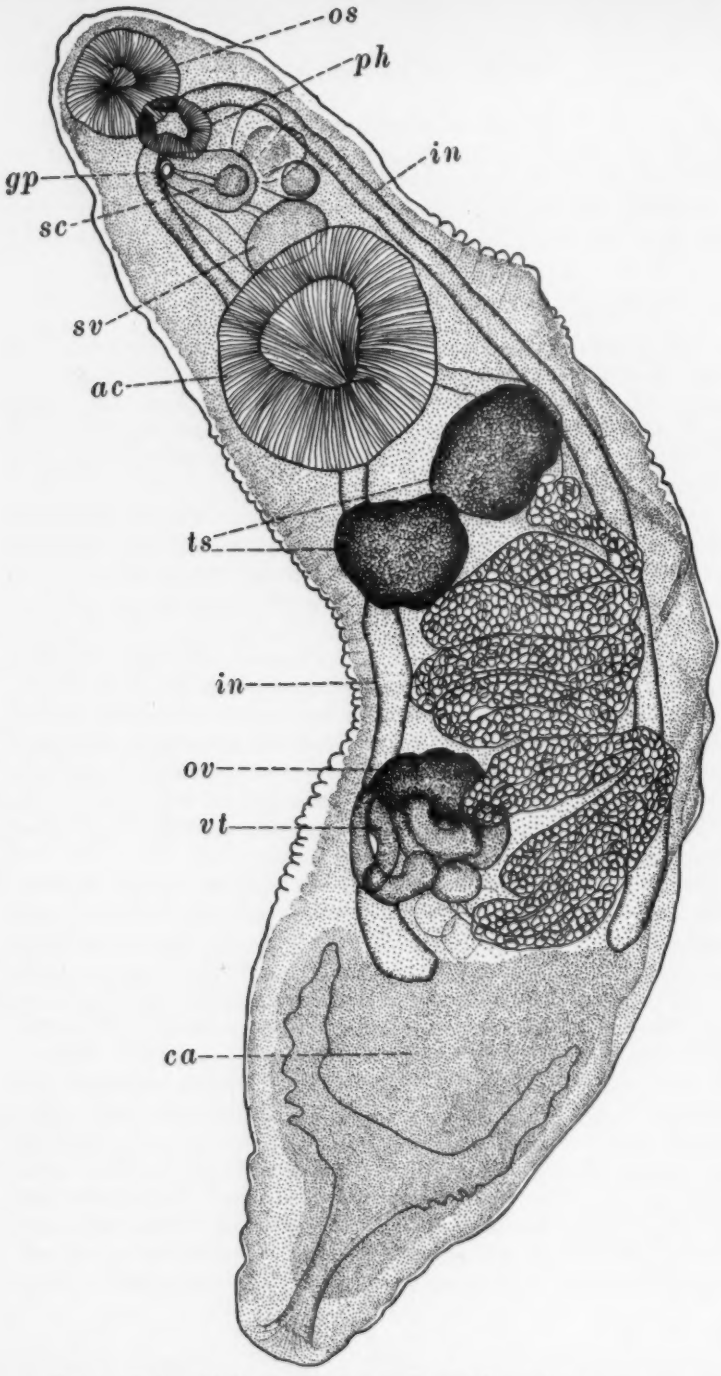

FIG. 1. *S. branchialis*, ventral view, specimen 1.8 mm. long.

Throughout its length the duct is surrounded by the cells of the prostate gland. The copulatory organs and sinus sac agree in structure with those of *S. musculus* as described by Looss.

Female Reproductive System. The ovary is globular, somewhat ventral and to the right of the midline, usually some distance behind the testes but may be pushed forward on retraction of the "tail." It is slightly broader than long, about the same size as one of the testes. The vitellaria are lobular, posterior and adjacent to the ovary; arranged in two groups united by a short slender bridge, four lobes on the right and three on the left. The lobes are sometimes indistinct, spherical to oval, and may be three times as long as wide with thickened ends. The oviduct arises at the posterior end of the ovary and turns ventrad to join the oötype where a short vitelline duct is received. Mehlis' gland is present, the seminal receptacle and Laurer's canal absent. The initial portion of the uterus is filled with spermatozoa. The uterus is much coiled; it passes at first posteriad for a short distance and then forward to join the metraterm. The eggs are very numerous, 0.016–0.021 mm. in length by 0.01–0.013 mm. in width; average of 100 eggs, 0.019 by 0.012 mm.

Copulatory Organs. The genital pore is median, immediately behind the oral sucker. The sinus sac is pyriform, directed obliquely to the dorsal and anterior face of the acetabulum, between the intestinal rami. It is filled with loose, vacuolated parenchyma and contains the hermaphroditic duct, prostate vesicle, and terminal part of the metraterm. The arrangement of the fibers in the wall was described by Looss, who compared this structure with the closed cirrus sac of other digenetic trematodes. The hermaphroditic duct extends two-thirds to three-fourths of the length of the sinus sac, is almost straight, and usually contains eggs. It is undoubtedly formed by the fusion of the distal portions of the metraterm and ejaculatory duct. From the inner end of the hermaphroditic duct the metraterm extends caudad as a strong, straight tube. Dorsally, the short ejaculatory duct opens into the prostate vesicle, a spherical organ situated at the base of the sinus sac. The vesicle may contain spermatozoa, although usually it is either empty or partially filled with droplets of secretion from the prostate cells.

Excretory System. The excretory vesicle divides behind the acetabulum, and the crura unite dorsal to the pharynx. Further details of the system could not be observed.

Comparisons

As pointed out by Looss (1907*b*), the ability of the hemiurid trematodes to retract the posterior part of the body and telescope it into the

major portion makes it exceedingly difficult to compare different species, and the criteria usually applied to other distomes can be utilized only with reservations. Very great changes in the shape and spatial relations of the internal organs are produced as the " caudal appendage " is protruded and retracted. These changes make it necessary to compare worms with similar degrees of contraction and frequently the limits of protrusion and retraction and corresponding internal changes can not be determined from fixed material alone. Only when an abundance of material is available can the observer distinguish with certainty between individual variations and specific distinctions. Comparison of specimens at different developmental stages is also helpful. Although specific determination is based primarily on morphological features, it is often facilitated by the biological characteristics of the specimens in question. Among parasitic species, the host-parasite relations have received much attention in recent years.

Sterrhurus branchialis differs in certain respects from each of the previously described species. Although other species have been found infrequently on the gills of their hosts, the chief seat of infestation is the stomach or adjacent portions of the digestive tract. In *S. branchialis,* conversely, the parasites occur primarily on the gills and were found in enormous numbers. Morphologically, *S. branchialis* is very similar to *S. musculus,* but the worms attain a larger size and the vitellaria are better developed. In Looss' figure of *S. musculus,* the vitelline glands are small, about twice as long as wide, the lobes are short, and their number was not stated.

S. branchialis is intermediate in size between *S. musculus* and *S. imocavus. S. imocavus* is slightly larger, more elongate, with larger suckers, smaller eggs, and less distinctly lobed vitellaria. Although the limits of body size overlap, *S. branchialis* differs from *S. grandiporus* in several respects. In *S. grandiporus* the suckers are relatively larger, the vitelline lobes are larger and longer, the " tail " is relatively smaller, and concomitant perhaps with the smaller " tail," more of the uterine coils are post-ovarian in position. *S. fusiformis* may be distinguished from

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Abbreviations in Fig. 2

ac—acetabulum
ca—caudal appendage
gp—genital pore
hd—hermaphroditic duct
in—intestine
os—oral sucker
ov—ovary
ph—pharynx
pr—prostate gland
pv—prostate vesicle
sc—sinus sac
sv—seminal vesicle
ts—testis
ut—uterus
vt—vitellaria

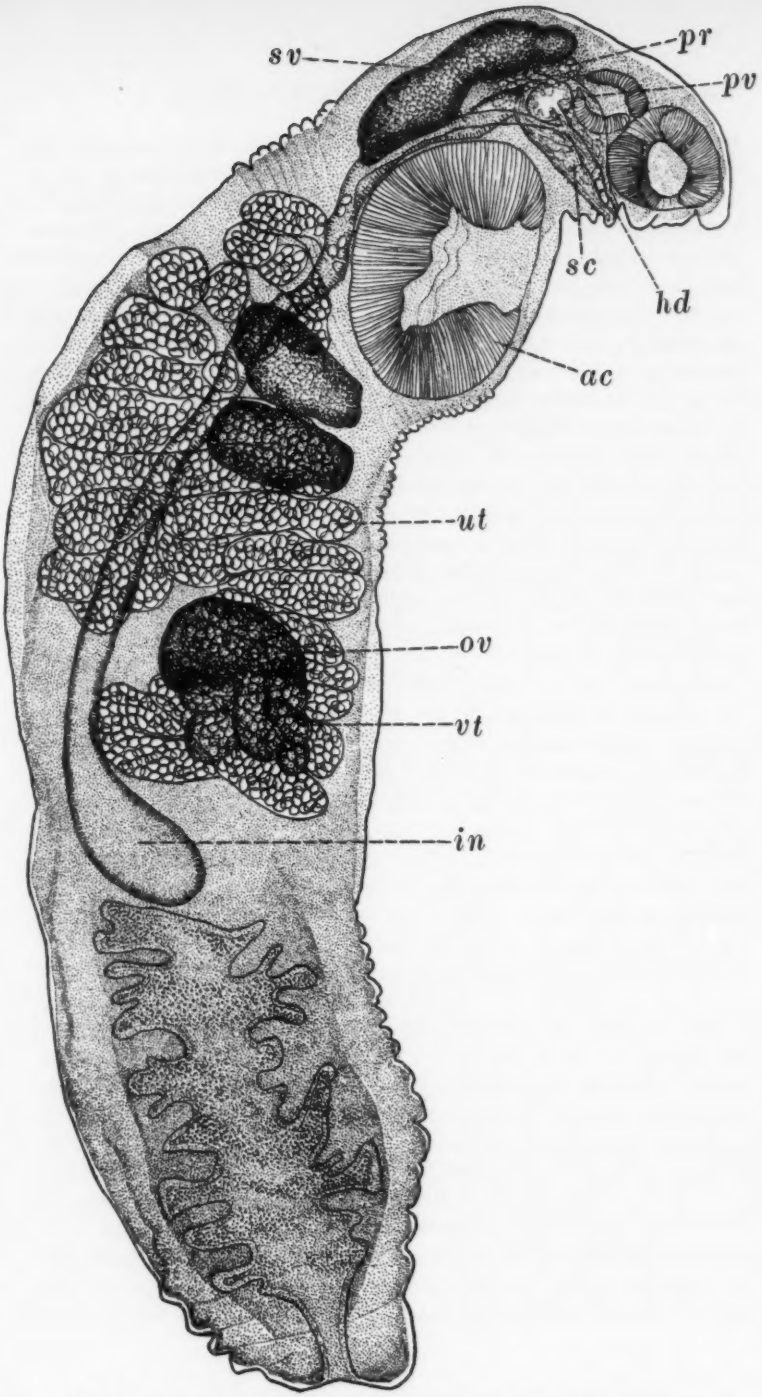

FIG. 2. *S. branchialis*, from right side, specimen 2.15 mm. long.

S. branchialis since in the former species the worms are larger and the vitelline lobes are much longer and thinner. More than one species has undoubtedly been described as *S. monticellii* and the name should be restricted to the form originally described by Linton (1898) from *R. remora*. These specimens are almost twice the size of *S. branchialis* and show corresponding differences in size of internal organs. Otherwise the two species are very similar. Certain of the small specimens referred by Linton to *S. monticellii* were assigned by Manter (1934) to the species which he described as *S. floridensis*. This species, according to the author, differs from *S. musculus* only in size of eggs. *S. lævis*, as redescribed by Manter (1934), differs from *S. branchialis* in form of vitellaria, seminal vesicle, and sinus sac. *S. præclarus* differs from *S. branchialis* in form of copulatory organs and vitellaria, location of testes, and size of caudal appendage. *S. robustus* differs from *S. branchialis* in the absence of a sinus sac and in the location and form of the seminal vesicle. *S. branchialis* may be distinguished from *S. profundus* by differences in the position of the anterior limits of the "tail," and in the form of the copulatory organs and vitellaria.

Discussion

As noted by Odhner (1911), the Hemiuridæ are very similar and probably closely related to the Azygiidæ. In all of these trematodes the musculature is well developed and specimens assume varied forms on contraction. According to Ward (1917), "This (*Azygia*) is a powerfully muscular distome and may be greatly distorted in the process of preservation. Specimens taken from a single host at the same time and preserved in the same way often present marked external differences in size and form. The genera *Megadistomum* of Leidy and Stafford, *Mimodistomum* of Leidy, and *Hassalius* of Goldberger are instances of such extreme specimens that really belong to the single genus *Azygia*." Indeed, the same condition was encountered in *S. branchialis*. Such great differences were found in the large number of worms studied that for a time it appeared that more than one species was represented. Different specimens, however, showed intermediate conditions and made it possible to assign all the material to a single species. This enormous variation among individuals of a single species makes it exceedingly difficult to distinguish between different species. Although they may be distinct and valid, we have attempted without success to formulate a satisfactory key to the several species of *Sterrhurus*.

In his diagnosis of the genus *Sterrhurus*, Looss (1907*b*) placed special emphasis on the form of the copulatory organs. He stated, "Der den Cirrusbeutel ersetzende Muskelsack umschliesst ausser dem Ductus

hermaphroditus auch den Anfangsteil des Metraterms und den kurzen Ductus ejaculatorius, der seinerseits in den blasenartigen, das Hinterende des Cirrussacks einnehmenden Hohlraum übergeht. In diesen tritt von hinten die ausserhalb des Sacks gelegene Pars prostatica ein, wobei ihr innerer Belag muttermundartig in die Blase vorspringt."

The species *S. robustus, S. præclarus,* and *S. profundus* of Manter have copulatory organs which do not conform to the generic diagnosis and *S. profundus,* according to Manter, " differs from all others in the genus in the extreme development of the tail, the undivided seminal vesicle, the far forward position of the genital pore, the lack of prostate gland, the straight ascending limb of the uterus and the apparently ununited branches of the excretory vesicle. Its unlobed vitellaria are unusual as is also the extreme posterior extent of the uterus and ceca." The inclusion of these species in the genus *Sterrhurus* was made with the following explanation, " There arises a question as to whether the genus should be limited to species with sinus sac and terminal genital ducts as in *Sterrhurus musculus* Looss (the type species) and *Sterrhurus floridensis* (figs. 69, 70) or whether it should include also such forms as *Sterrhurus robustus, Sterrhurus præclarus,* and *Sterrhurus profundus.* During the early development of any genus, it is difficult to arrive at a logical generic limitation. As more species of *Sterrhurus* are named, the genus will undoubtedly be divided. At present it seems best to allow the genus to grow, based on the fundamental characters of smooth body provided with a tail appendage rather than to erect several genera of single species."

In our opinion the form of the copulatory organs is a feature of generic rank, and a group based only on a " smooth body provided with a tail appendage " is not a genus but deserves subfamily rank. Accordingly, since the species in question have copulatory organs which differ so fundamentally from those of *S. musculus,* we would not include these species in the genus *Sterrhurus.*

Since we have not examined material of other species of *Sterrhurus,* opinion concerning their validity can be based only on the published accounts in the literature. Many of the descriptions are brief and authors do not always agree on the dimensions given for a single species. A discussion of this subject may be found in the recent paper by Manter (1934). For example, the great variation in the dimensions recorded by Linton for the several forms described by him at various times as *Distomum monticellii* and the lack of complete descriptions make it difficult to characterize that species. Linton's (1898) measurements and figure differ considerably from those given in (1910) when he redescribed the parasite and placed it in the genus *Sterrhurus.* The speci-

mens reported in 1898 are about four times as large as those described as *S. monticellii* in 1910. In his (1905) paper Linton described the parasite from a large number of hosts. His Fig. 154 was sketched from life with details added from fixed and stained specimens. The worm was taken from *Rachycentron canadus* at Beaufort, North Carolina. It was about the same size as the specimens described in 1898. The vitelline lobes as shown in this figure differ slightly from those of another specimen shown in Fig. 155 which had been taken from the sand pike, *Synodus fœtens*. It appears certain that more than one species must be represented in the material reported by Linton.

According to Manter (1934) the worms described as *D. monticellii* by Linton (1907) are probably specifically identical with *S. floridensis* Manter, 1934. But this species according to the author differs from *S. musculus* only in size of eggs. Looss gave the egg measurements of *S. musculus* as 0.019–0.021 mm. by 0.011–0.013 mm. The maximum egg length in *S. floridensis* is 0.017 and the greatest width about 0.01 mm. Most of the eggs measure from 0.013–0.015 mm. in length. The importance of egg measurements in determining specificity has been discussed by many authors. Cort (1915) noted the variation in egg size that may occur in a given species. In *S. branchialis* the egg measurements show variations that are close to the limits of all the described species. Size of eggs, in the absence of other morphological differences, is a very inadequate criterion by which to characterize a new species. This is true especially in cases where the life cycle is unknown. In the same species, e.g., *Fasciola hepatica* and *Clonorchis sinensis*, it has been demonstrated repeatedly that worms which have completed their development in different host species may show marked differences in size and in size of eggs. There is a further complicating possibility. It has been observed (see Stunkard, 1931) that trematode parasites of fishes may persist for a time in the digestive tract of piscivorous hosts and may become adapted to the new environment. Since the hosts of *Sterrhurus* feed in part at least on other fishes, such a method of disseminating the infestation can not be overlooked. In fact it is indicated by the fifty-five different species of fish which are listed by Manter as hosts of *S. floridensis*. An observation by Looss (1907*b*), "dass man bei jungen Sterrhurinen, die sich oft in Mengen encystiert im Peritoneum und anderen Organen verschiedener Fische finden," is of much significance in this connection. It indicates clearly that either different fishes normally serve as second intermediate hosts or that if ingested while young the parasites may encyst in these fishes awaiting a chance introduction into the proper host where they may complete their development. In the

absence of knowledge concerning the life cycle and larval stages of these trematodes, specific identification must be largely tentative.

BIBLIOGRAPHY

CORT, W. W., 1915. Egg Variation in a Trematode Species. *Jour. Parasitol.*, **2**: 25.

FUHRMANN, O., 1928. Trematoda. In: Handbuch der Zoologie von Kükenthal-Krumbach. Berlin u. Leipzig.

LINTON, EDWIN, 1898. Notes on Trematode Parasites of Fishes. *Proc. U. S. Nat. Mus.*, **20**: 507.

LINTON, EDWIN, 1901. Parasites of Fishes of the Woods Hole Region. *Bull. U. S. Fish. Comm. for 1899:* 405.

LINTON, EDWIN, 1905. Parasites of Fishes of Beaufort, North Carolina. *Bull. Bur. Fish.*, **24**: 321.

LINTON, EDWIN, 1907. Notes on Parasites of Bermuda Fishes. *Proc. U. S. Nat. Mus.*, **33**: 85.

LINTON, EDWIN, 1910. Helminth Fauna of the Dry Tortugas. II. Trematodes. *Carnegie Inst. Washington, Publ. No. 133*, p. 11.

LOOSS, A., 1899. Weitere Beiträge zur Kenntniss der Trematoden-Fauna Aegyptens, zugleich Versuch einer natürlichen Gliederung des Genus Distomum Retzius. *Zool. Jahrb., Abt. System.*, **12**: 521.

LOOSS, A., 1907*a*. Zur Kenntniss der Distomenfamilie Hemiuridae. *Zool. Anz.*, **31**: 585.

LOOSS, A., 1907*b*. Beiträge zur Systematik der Distomen. Zur Kenntniss der Familie Hemiuridae. *Zool., Jahrb., Abt. System.*, **26**: 63.

MANTER, H. W., 1931. Some Digenetic Trematodes of Marine Fishes of Beaufort, North Carolina. *Parasitol.*, **23**: 396.

MANTER, H. W., 1934. Some Digenetic Trematodes from Deep-water Fishes of Tortugas, Florida. *Carnegie Inst. Washington, Publ. No. 435*, p. 257.

NICOLL, W., 1915. The Trematode Parasites of North Queensland. III. Parasites of Fishes. *Parasitol.*, **8**: 22.

NIGRELLI, R. F., AND H. W. STUNKARD, 1933. Sterrhurus sp., a Hemiurid from the Gills of the Cutlass Fish (Trichiurus lepturus L.). *Jour. Parasitol.*, **20**: 136.

ODHNER, T., 1911. Zum natürlichen System der digenen Trematoden: IV. *Zool. Anz.*, **38**: 513.

STUNKARD, H. W., 1931. Further Observations on the Occurrence of Anal Openings in Digenetic Trematodes. *Zeitschr. Parasitenk.*, **3**: 713.

WARD, H. B., 1917. On the Structure and Classification of North American Parasitic Worms. *Jour. Parasitol.*, **4**: 1.

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